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The influence of Effective Microorganisms and number of buds per cane in viticulture on chemical composition in fruits

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Summary

As a result of climate warming, wine-growing zones have moved to the north, where conditions exist may result in poor fruit quality. Fruits may develop significant amounts of tannin compounds, which are not acceptable to all consumers. The aim of this study was to demonstrate the influence of selected factors on the quality and content of polyphenols in grapevine fruits. The differentiating factors were as follows: two grapevine cultivars, varied number of buds per cane, and treatment with Effective Microorganisms (EM). To determine the total content of polyphenols and individual polyphenolic compounds in the tested fruits, the UPLC-PDA-MS method was used. The results indicated that the studied factors had no effect on total soluble solids and titratable acidity in grapes. The experiment revealed that polyphenol content was most dependent on the cultivar, followed by the number of buds per cane; EM treatment had the least effect. The fruit of the 'Regent' cultivar was characterised by higher polyphenol content. 'Cabernet Cortis' berries had higher levels of phenolic acids and flavan-3-ols, while 'Regent' berries were higher in anthocyanins and flavonols. EM treatment had a large impact on the reduction of tannic acid compounds. Fruits from untreated plants with four buds per cane had a significantly increased content of polyphenols, including flavan-3-ols.

Key words: polyphenol content, grapevine, Effective Microorganisms, buds per cane, tannin

Introduction

Extensive chemisation of agricultural fields has resulted in progressive environmental pollution, and the agents applied have exerted negative effects on the human body. Therefore, natural cultivation methods and preparations are being sought to ensure both bountiful and high-quality harvests (Javaid and Bajwa, 2011). Effective Microorganisms (EM) are biological preparations that contain selected, naturally occurring microbes, such as *Lactobacillus casei*, *Rhodopseudomonas palustris*, *Saccharomyces albus*, *Streptomyces albus*, and *Aspergillus oryzae* (Higa, 1989; Hu and Qi, 2013). The microorganisms in the preparations are capable of high levels of antioxidant production, and as such, they naturally aid the plant protection system. The EM technology was developed by Teruo Higa in the 1980s. The concept of using microbiological preparations in agriculture and environmental protection is thought to be an environmentally friendly alternative to commonly applied chemical agents (Higa, 1989).

Grapevine pruning and shrub training with a specific number of buds is the basic ampelotechnical treatment aimed at yielding high-quality fruits in vineyards (Brighenti et al., 2017). Pruning creates favourable conditions for the setting of properly sized fruits that are the right colour and have appropriate solid content. In addition, the procedure prevents damage that can be caused by shrubs becoming overloaded with too many fruits (Senthilkumar and Vijayakumar, 2015).

Polyphenols are one of the most frequently studied groups of biologically active compounds due to their health-promoting properties. At a time when cancer and cardiovascular disease are increasingly on the rise, polyphenols are a natural adjuvant in the treatment, as well as prevention, of such diseases. The biological activity and medicinal properties of polyphenols are associated with the diversity of their structures. The compounds classified as polyphenols demonstrate high antioxidant, anti-inflammatory, antibiotic, and anticarcinogenic activity (Mijowska et al., 2016).

The most frequently mentioned sources of this valuable group of compounds include red grapes and their processed products, primarily wines (Mijowska et al., 2017a). According to the International Organisation of Vine and Wine's 2017 statistical report on world vitiviniculture, the harvested output is approximately 77 million tons a year worldwide, of which 36% is consumed as fresh fruit and 47% is dedicated to wine production.

The polyphenol content in individual parts of the grapevine fruit is variable; the highest value is in the seeds at 60%-70%, with 28%-35% in the skin and approximately 10% in the fruit pulp (Godjevac et al., 2010). The structures of phenolic compounds in grapes have a significant effect on the formation of flavour and the aromatic properties of wines, as well as their colour, whereas anthocyanins play the most important role in the colour of grapes and wines (He and Giusti, 2010). The composition of the polyphenolic profile in grapes depends on many factors, such as the vine cultivar, degree of fruit ripeness, climatic and soil conditions, and physiological state of the plant. Interactions among the aforementioned agents make it difficult to isolate a specific effect of one agent on polyphenol content. Therefore, scientists and winemakers are unceasingly conducting research to investigate these factors (Pantelić et al., 2016).

From the consumer's point of view, a higher content of polyphenols is beneficial for health reasons. However, due to the wines taste properties, it would be advisable to obtain fruit with a reduced flavan-3-ol content. The aim of this experiment was to examine the effect of EM and the number of buds per cane on the quality of fruit grown in north-western Poland, including the content and profile of polyphenols. The study also investigated the relationships between the studied factors, which of the cultivation methods most effectively reduced the content of tannic compounds.

Material and methods

Location

The analysed material came from a non-irrigated five-year-old vineyard at the Research Station of West Pomeranian University of Technology in Szczecin. The site is in the north-western part of Poland called the Szczecin Lowland, approximately 90 km from the Baltic Sea. In this area, there are numerous hills the remnants of

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the frontal moraine that are 20-60 m above sea level. These hills affect the distribution and intensity of rainfall, number of sunlight hours, temperature, and wind speed. The climate is also significantly affected by the presence of large water basins (Szczecin Lagoon, Dąbie Lake, and the Odra River), which provide additional moisture during the vegetation period. The majority of the West Pomeranian Province belongs to zone 7A on Heinz and Schreiber's 'Map of zones of plant resistance to frost'. However, in Szczecin and in the nearby northern region, minimum temperatures range from -12 °C to -15 °C, which correspond to values typical of zone 7B (MIJOWSKA et al., 2016).

Tab. 1 indicates changes in the weather for the years 2014 and 2015, as well as significant deviations from the average growing season during the multi-year period from 1951 to 2012. During the 2014 and 2015 growing seasons, average temperatures and sun hours were similar. Unusually low precipitation was observed during the 2015 growing season (242 mm), compared to the years 1951-2012 and 2014 (391 and 448 mm, respectively). The period from April to July 2015 was characterised by lower average temperatures and less sun hours relative to the same period in 2014. The largest weather deviation in 2015 took place in August, when rainfall was extremely low (14.7 mm) compared to the average rainfall for the growing season during the multi-year period and in 2014 (74.2 and 104.6 mm, respectively) (MIJOWSKA et al., 2016).

The orchard contained an agricultural soil with a natural profile, developed from silt loam with an unusually low density of 1.12 Mg m^{-3}, a pH of 6.7, and a high water capacity of 31.3% (w w^{-1}). It also contained a high level of organic matter (29.2 g kg^{-1} soil). Regardless of the site, the soils were characterised by similar low salinity (EC 0.37 mS cm^{-1}). In comparison to optimal soil mineral content as determined by SADOWSKI et al. (1990), the soil in which the plants were grown, regardless of the stand, was characterised by high levels of phosphorus at 84 mg kg^{-1} (optimum 20-40 mg kg^{-1}), potassium at 93 mg kg^{-1} (optimum 50-80 mg kg^{-1}), and magnesium at 44 mg kg^{-1} (optimum 25-40 mg kg^{-1}). Every spring, nitrogen fertilisation was applied at a dose of 60 kg N per hectare, and a calcium fertiliser was applied at a dose of 80 kg Ca per hectare.

Grape samples

Two red grapevine cultivars, 'Regent' and 'Cabernet Cortis', were included in this research. The vines were planted with a north-south row orientation at 1 × 2.6 m and pruned with a Guyot (one-cane) training system. During cultivation, plants were treated with EM1, a beneficial microorganisms and molasses solution prepared according to the manufacturer's instructions. EM1 stock solution was diluted 1:1,000 (EM:water) and applied in spray form every second week, starting at the appearance of leaf buds and continuing until harvest. Additionally, the cultivars were grown with either four or eight buds per cane. The berry samples were collected in 2014 and 2015 at technological maturity.

Sampling was performed by picking grape berries randomly distributed throughout both cultivars. Each sample consisted of three replications each of 100 randomly selected berries on both sides of the canopy and from different parts of the clusters. The collected samples were immediately frozen at -25 °C until analysis.

Physicochemical parameters

Total soluble solid (TSS) content of grapes was determined as degrees Brix (°Bx) using a PAL-1 (Atago, Tokyo, Japan) refractometer. Titratable acidity (TA) was determined by titration of a water extract of juice with 0.1 N NaOH to an end point of pH 8.1.

Extraction procedure

Grape berries were prepared according to the method described in OSZMIAŃSKI et al. (2013). The thawed berries were separated with methanol acidified with 2.0% formic acid. The separation was conducted two times by incubating the samples for 20 min under sonication (Sonic-6D, Polsonic, Warsaw, Poland), followed by shaking every 4 min. Subsequently, the suspension was centrifuged at 19,000 g for 10 min. Prior to the analysis, the supernatant was additionally purified with a 0.20 µm hydrophilic PTFE membrane (Millex Samplicity Filter, Merck).

The polyphenol content in each extract was determined by means of the ultraperformance liquid chromatography-photodiode array detector-mass spectrometry (UPLC-PDA-MS) method. All separations were conducted three times.

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

Analyses were performed according to the method described by MIJOWSKA et al. (2016). In fruit extracts for both cultivars, polyphenols were identified using an ACQUITY UPLC System appointed with a binary solvent manager, a PDA detector (Waters Corporation, Milford, MA, USA) and a G2 Q-T of micro mass spectrometer (Waters Corporation, Manchester, UK) equipped with an electrospray ionisation source operating in the negative and positive modes. Individual polyphenols were separated using a UPLC BEH C18

Tab. 1: Weather conditions during the vegetative season (April-October) in the years 2014-2015 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**
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**
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
	Sun hours							**Total**
2014	210	213	189	224	123	133	99	1191
2015	136	161	159	197	278	126	131	1188

column (1.7 µm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA, USA). Based on the data obtained, software was developed to scan multiple samples for defined substances. The various data analyses 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 a wavelength range of 200 - 600 nm in 2 nm increments. Finally, the retention times and spectra were compared with authenticated standards.

Statistical analysis

Analysis of variance (ANOVA) was carried out in order to estimate the influence of three different factors (cultivar, number of buds, and EM) on the physicochemical attributes of grapes. This method also allowed the statistical significance of the physical effect of particular factors, measured as contribution percentage, to be calculated (Davim and Reis, 2003). Mean comparisons were performed using Tukey's least significant difference (LSD) test with significance set at $p < 0.05$. The statistical analyses were performed using the Statistica 12.5 software.

Tab. 2: ANOVA table for total soluble solids and titratable acidity.

Source of variance	Total soluble solids p	Total soluble solids P (%)	Titratable acidity p	Titratable acidity P (%)
a	0.943	0.06	0.581	3.86
b	0.966	0.02	0.937	0.08
c	0.720	1.56	0.937	0.08
a × b	0.579	3.79	0.987	0.00
a × c	0.943	0.06	0.861	0.38
b × c	0.874	0.30	0.715	1.67
a × b × c	0.579	3.79	0.836	0.53
Error		90.42		93.40
Total		100.00		100.00

a – cultivar; b – number of buds per cane; c – EM treatment; p – probability of error, P (%) – percentage of contribution

Results and discussion

Total soluble solids (TSS) and titratable acidity (TA)

Statistical analysis demonstrated that the performed treatments (i.e., the number of buds per cane and EM treatment) had no significant effect on TSS (Tab. 2). The average TSS content in the fruits of both cultivars ranged from 16.85°Bx to 17.95°Bx, with the TA ranging from 0.525 to 0.640 mg 100 g^{-1} (Tab. 3).

TSS and TA are very important parameters in evaluating the usefulness of the fruit in processing. In Poland's climatic conditions, fruits of the cultivated vine cultivars contain 18% sugar on average, representing approximately 22% of TSS (Angelov et al., 2015).

Leão et al. (2016) concluded that the cultivar selected determines the method of shrub training (i.e., the number of buds per cane). Gladstones (1992) demonstrated that fruits that develop on vines with loose crowns feature a higher concentration of sugar in the juice and a better acid balance, unlike fruits that develop in shaded crown conditions. The results obtained in this experiment indicated that increased crown density had no significant effect on TSS and TA.

Polyphenols

The identification of 36 compounds categorised as anthocyanins, phenolic acids, flavanols, and flavan-3-ols was based on a comparison of their retention times, MS data, and MS/MS data with available standards and published data (Tab. 5-6). The research indicated that the studied factors had a significant effect on the total content of polyphenols in fruits (Tab. 4). The most significant factor that differentiated polyphenol content was the cultivar (P = 69.8%), followed by the number of buds (P = 10.8%) and the use of EM (P = 7.9%). Significant interaction was demonstrated between the ampelotechnical treatments applied for both vine cultivars. A significantly lower polyphenol content was found in fruits from plants treated with EM and trained with eight buds per cane 382.4 mg 100 g^{-1} of fresh weight (FW) for 'Regent' and 317.29 mg 100 g^{-1} FW for 'Cabernet Cortis', as compared to those from plants in the control conditions and rooted with four buds per cane (528.73 mg 100 g^{-1} FW for 'Regent' and 382.42 mg 100 g^{-1} FW for 'Cabernet Cortis') (Tab. 5). For vines rooted with four buds, as compared to vines with eight buds, the average total polyphenol content was higher by approximately 10.4% for 'Regent' and 11.4% for 'Cabernet Cortis'. The error value (P = 10.2%) indicates the magnitude of the effect exerted by other untested external factors on the measured parameters (Tab. 4).

The 'Regent' cultivar features a significantly higher total content of polyphenols in fruits (472.01 mg 100 g^{-1} FW), as compared to the 'Cabernet Cortis' cultivar (349.83 mg 100 g^{-1} FW), regardless of the treatments performed (Fig. 1). These values were more than twice as high as those of the fruits of 'Cabernet Sauvignon' (2356 mg kg^{-1}) and 'Tempranillo' (1489 mg kg^{-1}) cultivated in southern Europe (Guerrero et al., 2009). The quantity of polyphenols and

Tab. 3: Effect of the performed treatments on the total soluble solids (in °Bx) and titratable acidity (in g L^{-1}) in the fruit of the tested vine cultivars. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level. Lowercase letters (a) indicate group means; capital letters (A) indicate group averages.

	'Regent'		average	'Cabernet Cortis'		average
	C	EM		C	EM	
	Total soluble solids (°Bx)					
4	16.45 a	17.65 a	**17.05 A**	18.15 a	17.65 a	**17.90 A**
8	18.05 a	17.85 a	**17.95 A**	15.85 a	17.85 a	**16.85 A**
average	**17.25 A**	**17.75 A**		**17.00 A**	**17.75 A**	
	Titratable acidity (g L^{-1})					
4	0.595 a	0.660 a	**0.627 A**	0.580 a	0.585 a	**0.525 A**
8	0.645 a	0.620 a	**0.632 A**	0.600 a	0.580 a	**0.590 A**
average	**0.620 A**	**0.640 A**		**0.590 A**	**0.583 A**	

C – control treatment; EM – treatment with EM; 4,8 – number of buds per cane

Tab. 4: ANOVA table for polyphenol content.

Source of variance	Polyphenols p	Polyphenols P (%)	Anthocyanins p	Anthocyanins P (%)	Phenolic acids p	Phenolic acids P(%)	Flavonols p	Flavonols P(%)	Flavan-3-ols p	Flavan-3-ols P(%)
a	0.000	69.82	0.000	87.87	0.000	28.46	0.000	52.79	0.000	28.56
b	0.000	10.79	0.000	3.09	0.394	0.55	0.036	1.89	0.000	41.42
c	0.000	7.85	0.001	2.65	0.002	8.79	0.000	26.22	0.001	8.56
a × b	0.474	0.22	0.116	0.48	0.000	43.20	0.636	0.09	0.110	1.55
a × c	0.122	1.09	0.009	1.47	0.739	0.08	0.000	9.57	0.034	2.85
b × c	0.875	0.01	0.555	0.06	0.295	0.83	0.410	0.27	0.023	3.32
a × b × c	0.898	0.01	0.579	0.06	0.337	0.69	0.849	0.01	0.544	0.21
Error		10.20		4.31		17.39		9.16		13.53
Total		100.00		100.00		100.00		100.00		100.00

a – cultivar; b – number of buds per cane; c – EM treatment; p – probability of error; P (%) – percentage of contribution

Tab. 5: Effect of the performed treatments on polyphenol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

	'Regent' C	'Regent' EM	average	'Cabernet Cortis' C	'Cabernet Cortis' EM	average
	Polyphenols (mg 100 g^{-1} FW)					
4	528.73 a	469.98 ab	**499.35 A**	382.42 cd	357.01 cde	**369.71 C**
8	471.28 ab	418.03 bc	**444.66 B**	342.57 de	317.29 e	**329.93 D**
average	**500.01 A**	**444.00 B**		**362.50 C**	**337.15 C**	
	Anthocyanins (mg 100 g^{-1} FW)					
4	374.28 a	333.06 bc	**353.67 A**	206.38 d	207.50 d	**206.94 C**
8	338.81 ab	297.14 c	**317.98 B**	198.01 d	184.77 d	**191.39 C**
average	**356.55 A**	**315.10 B**		**202.20 C**	**196.14 C**	
	Phenolic acids (mg 100 g^{-1} FW)					
4	23.13 a	22.18 a	**22.66 A**	36.37 c	33.14 cd	**34.76 C**
8	30.83 bcd	26.34 ab	**28.59 B**	29.02 bc	25.64 ab	**27.33 B**
average	**26.98 AB**	**24.26 A**		**32.69 C**	**29.39 BC**	
	Flavonols (mg 100 g^{-1} FW)					
4	24.33 a	15.62 b	**19.98 A**	13.73 bc	11.34 c	**12.54 B**
8	22.06 a	14.60 bc	**18.33 A**	12.28 bc	10.67 c	**11.47 B**
average	**23.20 A**	**15.11 B**		**13.00 BC**	**11.01 C**	
	Flavan-3-ole (mg 100 g^{-1} FW)					
4	106.99 a	99.11 a	**103.05 A**	125.94 c	105.02 a	**115.48 C**
8	79.58 b	79.94 b	**79.76 B**	103.27 a	96.21 a	**99.74 A**
average	**93.28 AB**	**89.53 A**		**114.60 C**	**100.62 B**	

their composition in fruits depends on the cumulative parts (i.e., peel, flesh, and seeds). This is characteristic for a given cultivar (PANTELIĆ et al., 2016).

As indicated in this experiment, the better lighting conditions of vines rooted with a smaller number of buds resulted in a higher polyphenol content in the fruits. This corresponds to the findings of other authors (DEGU et al., 2016; HASELGROVE et al., 2000).

Due to the process-related usefulness of the yield, the content of polyphenol compounds is one of the most important parameters of quality in grapes and wines. Polyphenols have a direct impact on the organoleptic characteristics of wines, such as taste, sourness, bitterness, and colour (GARRIDO and BORGES, 2013). They are also strong antioxidants, which are valuable to human health. Therefore, the results obtained after treatment with EM contradict the expectations of both producers and consumers. The use of EM in cultivation caused a significant decrease in total polyphenol content in grapes, regardless of the selected cultivar and type of pruning. From a physiological point of view, this reduction creates favourable conditions for plants. EM is a natural protective preparation designed to relieve physiological plant stress caused by various external factors (HIGA, 1989; TALAAT, 2014). However, increased levels of antioxidants in plants often indicate initiation of defence processes as a response to the intensified action of free radicals (TALAAT et al., 2015).

Anthocyanins

Anthocyanins were the largest group of polyphenolic compounds analysed in the fruits of both cultivars (Tab. 6-7). Anthocyanins, classified as a flavonoid subgroup, are natural plant pigments that may appear mainly as violet, dark blue, and red. Anthocyanin accumulation in grape skins was significantly higher at 20 °C than

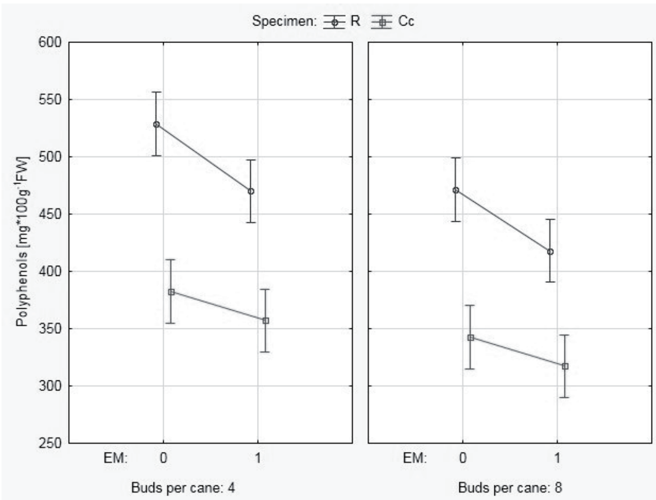

Fig. 1: Effect of the performed treatments on total polyphenol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

at 30 °C, and the most temperature-sensitive stage was from one to three weeks after colouring began (YAMANE et al., 2006).
The analysis of variance showed that the total content of anthocyanins was significantly affected by the cultivar, the number of buds per cane, and EM treatment, as well as the interaction between the number of buds and EM treatment (Tab. 4). The cultivar was found to have the highest percentage share (87.9%) of influence. The shares for the other factors (i.e., EM and the number of buds) were 2.65% and 3.1%, respectively. As in the case of total polyphenols, the error value (P = 4.3%) was higher than the P values for EM treatment and the number of buds; this indicates that untested factors had a greater influence on anthocyanin content.
Apart from its bioactive properties, anthocyanins are particularly important in the technology of red wine production (MIJOWSKA et al., 2017a). For the tested grapes, 19 groups of these compounds were identified. In fruits of the 'Regent' cultivar, the compounds amounted to more than 70% and, for 'Cabernet Cortis', 53.8% to 58.2% of all polyphenolic compounds (Tab. 6-7). The most abundant anthocyanins in the 'Regent' cultivar fruits were the 3-*O*-glucoside forms of petunidin, peonidin, delphinidin, malvidin, and cyanidin. These compounds are among the most important anthocyanins in grapes (IVANOVA et al., 2010). According to other authors, malvidin derivatives are the largest group of anthocyanin compounds in red grape cultivars (FIGUEIREDO-GONZÁLEZ et al., 2012). The total anthocyanin content in the 'Regent' cultivar fruits was 335.83 mg 100 g^{-1} FW and, for 'Cabernet Cortis', 199.17 mg 100 g^{-1} FW (Fig. 2). They represented 71.2% and 56.9%, respectively, of the polyphenol content in fruits of the tested vine cultivars. For the 'Regent' cultivar, the highest quantity of anthocyanins (374.28 mg 100 g^{-1} FW) was found in the fruits of vines rooted with four buds per cane and in controlled conditions. A significant decrease was observed after EM treatment: from 338.81 to 297.14 mg 100 g^{-1} FW with eight buds and from 374.27 to 333.06 mg 100 g^{-1} FW with four buds (Tab. 6). For 'Cabernet Cortis', the performed treatments produced no significant changes in anthocyanin content (Tab. 6).
Environmental factors have a greater influence on the levels of anthocyanins than on their composition, which is more closely related to the vine cultivar. Anthocyanins are the main cause of the red colour of fruits, and they must be extracted from such fruits. They are accumulated primarily in the fruit skin and, in some cultivars, also in the flesh (FLAMINI et al., 2013). This study confirms that anthocyanin composition is characteristic of the cultivar, as determined by the research conducted by other authors.

Shrubs with different numbers of buds feature different crown microclimates. The type of pruning applied results in changes in the levels of solar radiation, temperature, humidity, and wind (SMART, 1987), affecting the composition and quality of grapes. In the completed experiment, thinning out the vine crown had a positive effect on the content of anthocyanins in fruits. Similar results were obtained by ASELGROVE et al. (2000) and MIJOWSKA et al. (2016).
According to TALAAT (2014), EM preparations support the detoxification mechanism in plants exposed to stress. In plants subjected to EM, an increased ability to sweep H_2O_2 in the glutathione-ascorbate cycle was observed. This can effectively attenuate the plants' defensive reaction to stress. In this research, the Cabernet Cortis cultivar exhibited similar behaviour after EM treatment. However, from the producer's and consumer's viewpoints, a lower anthocyanin content in fruits is undesirable.

Phenolic acids

The profile of phenolic acids in the fruits of the studied cultivars was diversified and depended on the agrotechnical treatments performed. Phenolic acids represented 5.4% of the total polyphenol content for the 'Regent' cultivar and 8.9% for the 'Cabernet Cortis' cultivar. The cultivar, EM treatment, and the interaction between the cultivar and the number of buds per cane had a significant effect on the content of phenolic acids (Tab. 4). The greatest influence was exerted by the interaction between the cultivar and the number of buds (P = 43.2%), followed by the cultivar (P = 28.5%) and treatment with EM (P = 8.8%).
Pruning the 'Regent' cultivar with eight buds in controlled conditions increased the phenolic acid content in the fruits. In turn, pruning the 'Cabernet Cortis' cultivar with a higher number of buds per cane and treating with EM resulted in a significant decrease in the content of phenolic acids (Tab. 5-6). The average concentration of phenolic acids in both vine cultivars was determined by the number of buds per cane, not by treatment with EM. For the 'Regent' cultivar with eight buds, significantly higher levels of phenolic acids were found, while a comparable result was obtained for 'Cabernet Cortis' vines with four buds per cane (Fig. 3). Similar relationships were observed by MIJOWSKA et al. (2016), who demonstrated that less shading of the shrub crown significantly decreased the phenolic acid content in the 'Regent' cultivar. EHRHARDT et al. (2014) found that the level of trans-caftaric acid depended on the cultivation site, with 12.70 mg kg^{-1} FW in 'Regent' grapes harvested in Italy and a much higher value (35.98 mg kg^{-1} FW) in the colder climate of Germany. In the authors' own research, the results for the 'Regent' fruits ranged from 18.84 mg 100 g^{-1} FW (four buds plus EM treatment) to 26.32 mg 100 g^{-1} FW (eight buds).

Flavonols

Flavonols are yellow pigments masked by anthocyanins in red wines; however, the pigments affect their colour by co-pigmentation (CASTILLO-MUÑOZ et al., 2010). As a result, the extraction of anthocyanins during wine production is enhanced (CASTILLO-MUÑOZ et al., 2007). The greatest biosynthesis of flavonols is observed during the intensive ripening of fruits that occurs post-veraison (MATTIVI et al., 2006).
In the fruits of the 'Regent' and 'Cabernet Cortis' cultivars, flavonols represented 4.1% and 3.4%, respectively, of the total polyphenols detected. The analysis of variance revealed that the performed treatments had a significant impact on the flavonol content in fruits. An interaction was observed between the cultivar and EM treatment (Tab. 4-5). Once again, the most crucial factor in determining the content of flavonols was the vine cultivar (P = 52.8%), followed by treatment with EM (P = 26.2%) and the interaction (P = 9.6%). The number of buds per cane (P = 1.9%) had a small but significant

Tab. 6: Polyphenol content (in mg 100 g^{-1} FW) of grape cultivar ‘Regent’. Means with same letter were not significantly different by Tukey’s comparison at $p < 0.05$ level.

	R8	R4	R8 EM	R4 EM
Compounds (mg 100 g^{-1})				
Delphinidin 3.5-diGlc	5.39 ab	6.33 b	4.79 a	5.67 ab
Delphinidin 3-O-Glc;Petunidin 3.5-diGlc	147.08 ab	161.01 b	127.64 a	142.45 ab
Peonidin 3.5-diGlc;Malvidin 3.5-diGlc;Cyanidin 3-O-Glc	112.78 ab	136.19 c	97.93 a	117.23 bc
Petunidin 3-O-Glc	21.27 b	20.78 b	13.68 a	11.09 a
Peonidin 3-O-Glc	16.22 b	13.58 a	12.88 a	13.75 a
Malvidin 3-O-Glc	22.25 a	20.53 a	27.94 b	28.74 b
Delphinidin 3-O-acetyl-Glc	0.90 a	1.39 b	0.79 a	1.23 b
Delphinidin 3-O-caffeoyl-Glc	0.71 ab	0.95 c	0.62 a	0.84 bc
Petunidin 3-O-acetyl-Glc	0.51 ab	0.64 c	0.45 a	0.56 bc
Cyanidin 3-O-caffeoyl-Glc	0.11 ab	0.13 b	0.10 a	0.11 ab
Petunidin 3-O-caffeoyl-Glc	0.44 ab	0.57 c	0.39 a	0.51 bc
Malvidin 3-O-acetyl-Glc	0.24 ab	0.30 c	0.21 a	0.27 bc
Delphinidin 3-O-coumaroyl-Glc	3.28 b	3.68 c	2.89 a	3.25 b
Peonidin 3-O-coumaroyl-Glc	0.82 c	0.70 b	0.72 b	0.62 a
Peonidin 3-O-caffeoyl-Glc	0.11 a	0.34 b	0.10 a	0.30 b
Malvidin 3-O-caffeoyl-Glc	1.53 ab	1.82 c	1.35 a	1.61 bc
Cyanidin 3-O-coumaroyl-Glc	1.64 bc	1.72 c	1.44 a	1.52 ab
Petunidin 3-O-coumaroyl-Glc	0.07 ab	0.08 b	0.06 a	0.07 ab
Malvidin 3-O-coumaroyl-Glc	3.47 a	3.52 a	3.19 a	3.23 a
Anthocyanins sum	**338.81 b**	**374.27 c**	**297.14 a**	**333.06 b**
GRP (cis- and trans-isomers	3.32 c	2.77 ab	2.92 bc	2.45 a
Caftaric acid (cis- and trans-isomers)	26.32 c	19.35 a	22.37 b	18.84 a
Coutaric acid (cis- and trans-isomers	0.30 b	0.22 a	0.27 b	0.20 a
Fertaric acid	0.07 a	0.11 b	0.06 a	0.10 b
Galic acid	0.82 c	0.68 ab	0.72 b	0.60 a
Phenolic acids sum	**30.83 c**	**23.13 ab**	**26.34 bc**	**22.18 a**
Myricetin glucoside	4.50 b	5.18 c	3.95 a	4.58 b
Myricetin glucuronidec	0.82 b	0.75 b	0.39 a	0.45 a
Rutin	1.66 a	1.63 a	1.46 a	1.44 a
Quercetin-3-Oglucoside	1.10 ab	1.29 b	0.97 a	1.14 ab
Quercetin glucuronide	13.90 b	15.38 b	7.76 a	7.92 a
Quercetin	0.09 ab	0.11 b	0.08 a	0.09 ab
Flavonols sum	**22.06 b**	**24.33 b**	**14.60 a**	**15.62 a**
Dimer B1	1.10 b	0.66 a	2.08 c	0.58 a
Catechin	3.56 c	2.74 ab	3.13 bc	2.42 a
Dimer B2	11.26 ab	12.43 b	9.90 a	11.48 ab
Epicatechin	51.06 a	78.58 b	53.51 a	73.23 b
Galloylated dimer	4.51 ab	5.12 b	3.97 a	4.52 ab
Dimer B4	8.08 b	7.46 ab	7.35 ab	6.88 a
Flavan-3-ols sum	**79.58 a**	**106.99 c**	**79.94 a**	**99.11 b**
TOTAL	**471.29 B**	**528.73 C**	**418.03 A**	**469.98 B**

C – control treatment; EM – treatment with EM; 4,8 – number of buds per cane

influence (Tab. 4). After EM treatment, a significant decrease in flavonol content was found in the ‘Regent’ cultivar (Tab. 5-7, Fig. 4). As in the research by MIJOWSKA et al. (2016), this study found that the crown structure (pruning type) did not affect the content of flavonols in that cultivar. In turn, neither treatment procedure had a significant influence on flavonol content for the ‘Cabernet Cortis’ cultivar (Tab. 5).

The flavonol group includes, in particular, quercetin and its derivatives, myricetin, kaempferol, and isorhamnetin. The quantity of flavonols largely depends on the vine cultivar (LIANG et al., 2011). The ‘Regent’ fruits were more abundant in quercetin derivatives, including rutin, as compared to the fruits of the ‘Cabernet Cortis’ cultivar. Taking into account the significant decrease in flavonol content in the ‘Regent’ fruits after EM treatment and the previously mentioned reports by TALAAT (2014) relating to the detoxifying properties of the preparation, a hypothesis can be constructed that quercetin derivatives have a significant effect on the glutathione-ascorbate cycle in the plant. Moreover, this may indicate a significant

Tab. 7: Polyphenol content (in mg 100 g^{-1} FW) of grape cultivar 'Cabernet Cortis'. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

	Cc8	Cc4	Cc8 EM	Cc4 EM
Compounds (mg 100 g^{-1})				
Delphinidin 3.5-diGlc	4.78 c	3.41 ab	3.65 b	2.97 a
Delphinidin 3-O-Glc;Petunidin 3.5-diGlc	81.43 ab	94.52 c	75.29 a	89.21 bc
Peonidin 3.5-diGlc;Malvidin 3.5-diGlc;Cyanidin 3-O-Glc	49.78 b	50.89 b	46.66 a	56.07 c
Petunidin 3-O-Glc	14.21 c	10.21 a	11.87 ab	12.73 ab
Peonidin 3-O-Glc	5.16 a	7.37 b	5.05 a	9.45 c
Malvidin 3-O-Glc	22.30 b	18.22 a	23.97 b	18.68 a
Delphinidin 3-O-acetyl-Glc	3.90 b	4.61 c	3.21 a	3.82 b
Delphinidin 3-O-caffeoyl-Glc	1.24 a	3.38 b	1.11 a	1.32 a
Petunidin 3-O-acetyl-Glc	1.29 a	1.03 a	1.26 a	1.00 a
Cyanidin 3-O-caffeoyl-Glc	2.27 b	1.96 a	2.02 a	1.96 a
Petunidin 3-O-caffeoyl-Glc	0.14 a	0.19 b	0.14 a	0.15 a
Malvidin 3-O-acetyl-Glc	0.26 ab	0.29 b	0.22 a	0.25 ab
Delphinidin 3-O-coumaroyl-Glc	5.62 c	4.91 ab	5.18 b	4.88 a
Peonidin 3-O-coumaroyl-Glc	0.56 a	0.70 b	0.55 a	0.56 a
Peonidin 3-O-caffeoyl-Glc	0.19 b	0.15 a	0.19 b	0.16 a
Malvidin 3-O-caffeoyl-Glc	0.68 b	0.64 b	0.51 a	0.61 b
Cyanidin 3-O-coumaroyl-Glc	1.15 a	1.07 a	1.12 a	1.02 a
Petunidin 3-O-coumaroyl-Glc	0.11 a	0.18 b	0.11 a	0.10 a
Malvidin 3-O-coumaroyl-Glc	2.92 b	2.67 a	2.66 a	2.58 a
Anthocyanins sum	**198.01 ab**	**206.38 b**	**184.77 a**	**207.50 b**
GRP (cis- and trans-isomers	1.95 b	1.74 a	1.92 b	1.71 a
Caftaric acid (cis- and trans-isomers)	26.19 ab	33.82 c	22.85 a	30.67 bc
Coutaric acid (cis- and trans-isomers	0.41 a	0.42 a	0.39 a	0.37 a
Fertaric acid	0.05 a	0.05 a	0.08 b	0.04 a
Galic acid	0.42 b	0.34 a	0.41 b	0.35 a
Phenolic acids sum	**29.02 b**	**36.37 c**	**25.64 a**	**33.14 bc**
Myricetin glucoside	4.35 a	5.15 b	5.29 b	3.89 a
Myricetin glucuronidec	0.91 b	0.82 ab	0.48 a	0.80 a
Rutin	0.36 a	0.51 c	0.45 bc	0.38 ab
Quercetin-3-Oglucoside	0.52 a	0.55 a	0.50 a	0.48 a
Quercetin glucuronide	6.04 cd	6.65 d	3.87 a	5.74 bc
Quercetin	0.10 b	0.05 a	0.09 b	0.05 a
Flavonols sum	**12.28 ab**	**13.73 b**	**10.67 a**	**11.34 ab**
Dimer B1	1.42 b	1.44 b	1.28 a	1.30 a
Catechin	2.05 b	1.99 ab	1.59 a	1.85 ab
Dimer B2	11.56 a	13.11 b	10.30 a	11.12 a
Epicatechin	76.70 b	98.59 c	67.60 a	76.28 b
Galloylated dimer	7.37 a	9.19 b	9.28 b	8.61 b
Dimer B4	4.18 b	1.61 a	6.16 c	5.86 c
Flavan-3-ols sum	**103.27 ab**	**125.94 c**	**96.21 a**	**105.02 b**
TOTAL	**342.57 B**	**383.76 C**	**317.29 A**	**357.92 B**

Cc – 'Cabernet Cortis'; 4,8 – numbers of buds per cane; EM – treatment with EM.

effect of EM on quercetin derivatives. This hypothesis could also justify the strong correlation between the cultivar and EM treatment. Varying the number of buds in the experiment did not have a significant influence on the content of flavonols. Different results were obtained by SPAYD et al. (2002), who showed that well insolated fruits of the 'Merlot' cultivar contained almost 10 times the total concentration of flavonols as those harvested from shaded clusters. In addition, BAIANO et al. (2015), FENG et al. (2015), and MIJOWSKA et al. (2016) presented the beneficial effect of thinning out the vine crown on flavonol content. Considering the error value of P = 6.4%, the lack of influence of the number of buds on flavonol content during the experiment may have been caused by other external factors excluded from the research.

Flavan-3-ols

Flavan-3-ols are a group of tannin compounds that plentifully occur mainly in red grapes. They are the cause of the 'structure' of wine,

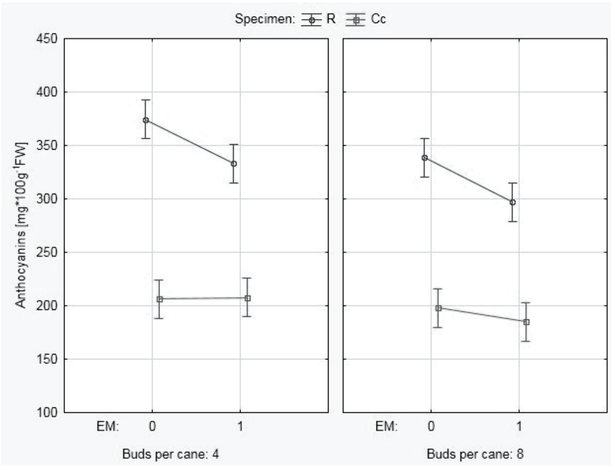

Fig. 2: Effect of the performed treatments on anthocyanin content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

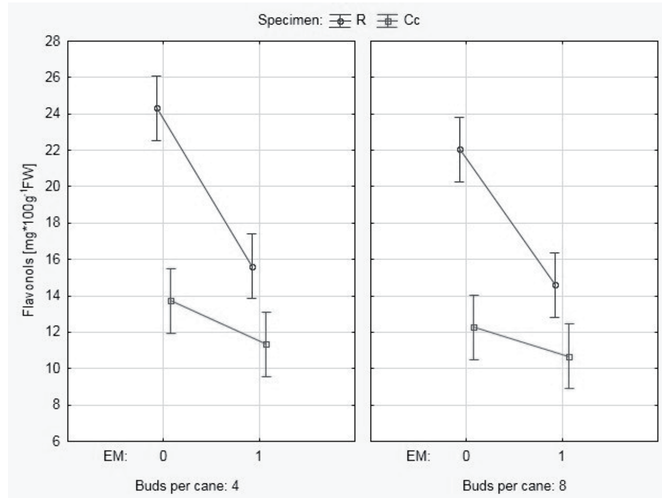

Fig. 4: Effect of the performed treatments on flavonol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

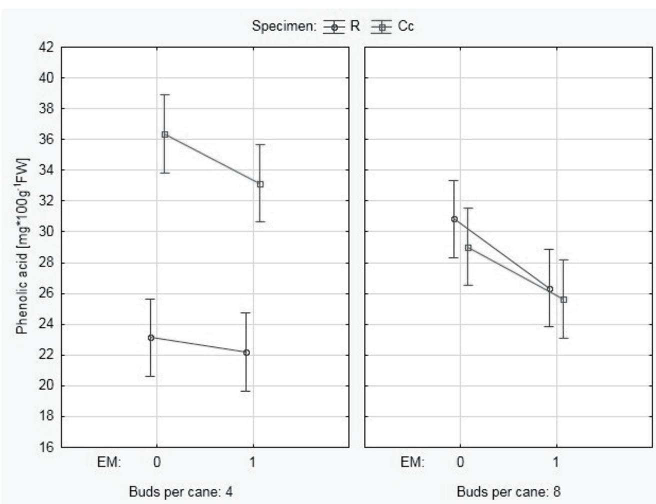

Fig. 3: Effect of the performed treatments on phenolic acid content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

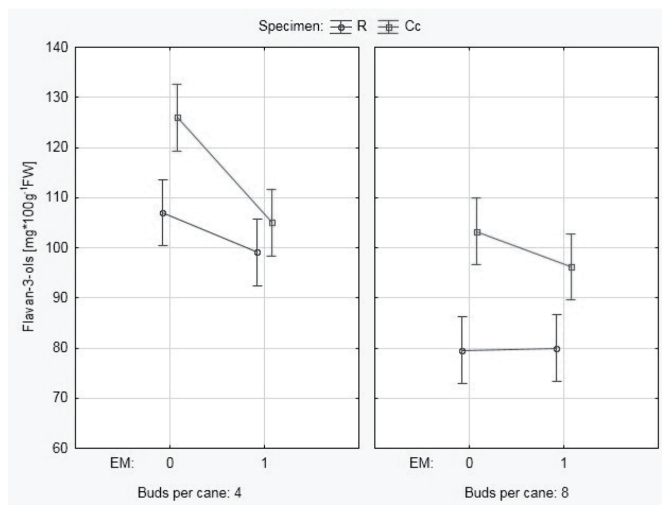

Fig. 5: Effect of the performed treatments on flavan-3-ol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

its astringency, and its bitterness. They also play a very important role in stabilising the red colour of wine (GUERRERO et al., 2009). During the maceration process, the compounds undergo extraction and pass on to the must. In wine prepared from the 'Regent' cultivar, flavan-3-ol content was approximately 70.4 mg 100 g^{-1} (MIJOWSKA et al., 2017b). This research found that the total content of flavan-3-ols in the 'Regent' fruits was 91.4 mg 100 g^{-1} FW, and 107.61 mg 100 g^{-1} FW for 'Cabernet Cortis'. Therefore, the compounds from this group represented 19.4% and 30.8%, respectively, of the total polyphenol content detected in the fruits of these vine cultivars. The analysis of variance demonstrated that total flavan-3-ol content was significantly influenced by the cultivar (P = 28.6%), the number of buds (P = 41.4%), EM treatment (P = 8.6%), and the interaction between pruning and EM treatment (P = 3.3%) as well as between the cultivar and EM treatment (P = 2.9%) (Tab. 4). In the fruits of both vine cultivars trained with a larger number of buds, the average flavan-3-ol content was significantly lower than the fruits of shrubs trained with fewer buds per cane (Fig. 5). EM treatment resulted in a significant decrease in flavan-3-ol content in the 'Cabernet Cortis' cultivar.

The content of flavan-3-ols in grapes depends on the cultivar; in the individual parts of the fruit, the content is highest in the seeds, followed in descending order by the skin, stick, and flesh (PANTELIĆ et al., 2016). Reduced shrub crown density contributed to an increase in flavan-3-ol content in fruits, as reported by MIJOWSKA et al. (2016) and REYNOLDS and VANDEN HEUVEL (2009). Furthermore, DEGU et al. (2016) concluded that light had no effect on the content of flavan-3-ols. As reported by LI et al. (2008), flavan-3-ols are recognised as the most sensitive of all flavonoids to non-enzymatic degradation processes. During fermentation, such compounds (e.g., catechins) may undergo partial splitting into phenolic units with a lower molecular mass. This process is mainly due to a temperature rise (GARRIDO and BORGES, 2013). A change in the quantity of these compounds was also observed during exposure to UVC radiation (MIJOWSKA et al., 2017a). The compounds of this group most frequently found in grapes are epicatechins, with levels ranging from 51.06 to 78.58 mg 100 g^{-1} FW for the 'Regent' cultivar, depending on the agrotechnical treatments applied. For 'Cabernet Cortis' fruits, the content was higher (67.60 to 98.59 mg 100 g^{-1} FW) (Tab. 6-7). The high content of the compound in fruits was confirmed by the research completed by EHRHARDT et al. (2014) and MIJOWSKA et al. (2017a).

DALY and STEWART (1999) concluded that EM treatment can improve growth and yield by increasing photosynthesis and producing bioactive substances, such as hormones and enzymes. Therefore, the reduction in flavan-3-ol content in fruits by means of EM may be justified, once again, by the antioxidant properties of the preparation.

Conclusion

The experiment demonstrated that the number of buds per cane and the use of EM had a significant effect on polyphenol content in the fruits of the wine grape cultivars studied. However, these factors had no significant effect on TA and TSS. The polyphenol content in the fruits was determined mainly by the cultivar and, to a lesser extent, the number of buds per cane and the smallest use of EM. The fruits of the 'Regent' cultivar were characterised by a higher polyphenol content compared to those of the 'Cabernet Cortis' cultivar. 'Cabernet Cortis' berries had higher levels of phenolic acids and flavan-3-ols, while those of 'Regent' had higher levels of anthocyanins and flavonols. Pruning plants with four buds per cane increased the content of all polyphenol groups studied, with the exception of phenolic acids in the 'Regent' cultivar. The use of EM reduced polyphenols, especially tannin compounds, in the fruits of both grape cultivars. From the point of view of wine production in a cold climate, this phenomenon is desirable and beneficial. Keeping the vines at four buds per cane without using EM mostly increased the levels of polyphenols, including the flavan-3-ols. However, leaving eight buds per cane and using EM contributed to a significant reduction of these compounds, as well as to a lower concentration of flavan-3-ols.
Selecting the proper cultivar and pruning the vine shrubs with a smaller number of buds per cane is conducive to obtaining fruits of higher quality and polyphenol content.

Author's contribution:
AA experimental design, field work and data collection, laboratory work, data analysis, redaction of manuscript; IO supervision, experimental design, data analysis; JW supervision, data analysis; JO laboratory work, data collection.

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