THE IMPACT OF CLUSTER THINNING ON FERTILITY AND BERRY AND WINE COMPOSITION OF 'BLAUER PORTUGIESER' (*VITIS VINIFERA* L.) GRAPEVINE VARIETY

Jan REŠČIČ*, Maja MIKULIČ-PETKOVŠEK, Franci ŠTAMPAR , Anka ZUPAN and Denis RUSJAN

[1]Chair for Fruit Growing, Viticulture and Vegetable Growing, Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Abstract

Aim: Two different yield reductions based on cluster thinning (CT) were performed to determine their impact on vine growth, yield, and grape and wine composition of 'Blauer Portugieser' grapevine variety.

Methods and results: Two levels of cluster thinning (limited CT1 – 20-30 % and severe CT2 – 40-50 % cluster reduction) were applied at the pea-size berry (BBCH 75) phenological stage in 2007, 2008 and 2011. The potential impact of CT was determined by measurements of vine growth and fertility potential, berry weight, berry colour, soluble solids content, titratable acidity, pH and total phenolics. Additionally, for the first time, individual phenolic compounds were identified and quantified in berry skin and wine by HPLC-MS. In general, CT of 'Blauer Portugieser' significantly decreased titratable acidity in grape and wine, and increased pH and chromatic parameters in grape and alcohol content and volatile acidity in wine. A significant decrease in yield per vine (of 0.92 kg of grape/vine), together with an increase in soluble solids (of 2.8 °Brix) in grape and pH and total extract content in wine was only observed in severe CT (CT2). Furthermore, CT2 significantly increased the content of total anthocyanins, flavonols and hydroxycinnamic acids, but not total flavanols, in grape and wine.

Conclusion: A significant impact on yield and grape and wine composition was observed, particularly in the CT2 treatment, in which the yield loss was compensated by higher contents of soluble solids in grape, alcohol in wine, and phenolic compounds in grape and wine.

Significance and impact of the study: The present study is the first report on the impact of different levels of cluster thinning on yield and grape and wine composition of 'Blauer Portugieser' variety. Grape and wine composition has been evaluated with an emphasis on a detailed profile of individual and total phenolic contents. The results are undoubtedly useful for winegrowers, who, until now, lacked technological guidelines to optimize 'Blauer Portugieser' yield and wine quality.

Key words: yield, grape quality, hydroxycinnamic acids, flavonols, anthocyanins, HPLC-MS

Résumé

Objectif : Deux réductions de rendement par éclaircissage ont été effectuées afin de déterminer leur incidence sur la croissance et le rendement des plantes ainsi que sur la composition du raisin et du vin de la variété 'Portugais Bleu'.

Méthodes et résultats : Deux niveaux d'éclaircissage (réduction de grappe limitée CT1 – 20-30 % et intense CT2 – 40-50 %) ont été effectués au stade phénologique BBCH 75 (grains de la taille d'un petit pois) en 2007, 2008 et 2011. L' incidence potentielle de l'éclaircissage a été déterminée par des mesures du potentiel de croissance et de fertilité des plantes ainsi que du poids et de la couleur des baies, de la concentration en solides solubles, de l'acidité titrable, du pH et des substances phénoliques totales. En outre, pour la première fois, les composés phénoliques individuels ont été identifiés et quantifiés par CLHP-SM dans la peau des baies et dans le vin. En général, l'éclaircissage du 'Portugais Bleu' a considérablement diminué l'acidité titrable du raisin et du vin, mais il a augmenté le pH et les valeurs des paramètres chromatiques du raisin ainsi que la teneur en alcool et en acidité volatile dans le vin.

Conclusion : Des incidences significatives sur le rendement et la composition du raisin et du vin ont été observées, en particulier suite à l'éclaircissage intense, où la perte de rendement a été compensée par une teneur en solides solubles plus élevée dans le raisin de même qu'une teneur plus élevée en alcool dans le vin et en composés phénoliques dans le raisin et dans le vin.

Signification et impact de l'étude : Cette étude rend compte pour la première fois de l'incidence de différents niveaux d'éclaircissage sur le rendement et la composition du raisin et du vin de la variété 'Portugais Bleu'. L'évaluation de la composition du raisin et du vin s'est concentrée sur le détail des teneurs en composés phénoliques individuels et totaux. Les viticulteurs qui, jusqu'à présent, manquaient d'orientations technologiques en vue d'optimiser le rendement et la qualité du vin de la variété 'Portugais Bleu' trouveront une utilité certaine dans ces résultats.

Mots clés : rendement, qualité du raisin, hydroxycinnamates, flavonols, anthocyanes, CLHP-SM

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*Corresponding author: jan.rescic@bf.uni-lj.si

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INTRODUCTION

Grape and wine production in Europe is an important and traditional agricultural sector, although its extent in EU has decreased by around 15 % in the past decades (O.I.V., 2013). This could be attributed to the high cost of grape production, especially in areas with small fragmented vineyards on high slopes where manual labour still predominates (Preszler *et al.*, 2010). Winegrowers are constantly trying to optimize the ratio between quantity and quality and to raise the price of wine. Yield and vegetative growth affect grape and wine quality and can be controlled by grape cluster and shoot thinning (Šuklje *et al.*, 2013), berry thinning (Gil *et al.*, 2013), shoot topping and defoliation after blooming (Reynolds *et al.*, 2004; Šuklje *et al.*, 2013). Cluster thinning (CT) is undoubtedly one of the most commonly used vineyard practices for achieving the optimal ratio between yield and quality (Naor *et al.*, 2002; Prajitna *et al.*, 2007; Preszler *et al.*, 2010), balancing crop and improving grape and wine composition (Naor *et al.*, 2002). Entire clusters or their parts are usually removed by hand at different phenological stages during grape ripening before harvest (Prajitna *et al.*, 2007; Di Profio *et al.*, 2011; Gil *et al.*, 2013). Although this is a costly and labour-intensive procedure (time-consuming and requiring skills, experience and precision), the enhanced product quality makes it economically feasible (Preszler *et al.*, 2010). However, timing and intensity of cluster thinning depend on the purpose and goal of the wine style. Prajitna *et al.* (2007) demonstrated that cluster removal at the pea-size stage significantly increased pH, total phenolic content and anthocyanins and decreased titratable acidity in grape. Bubola *et al.* (2011) reported that in 'Merlot' yield reduction by 60 % CT at véraison increased leaf area/fruit weight ratio from 1.96 m^2/kg in the control treatment to 2.08 m^2/kg. They demonstrated that CT significantly increased the amount of total phenolics by 510 mg/L and anthocyanins by 182 mg/L, compared to the control vines. Furthermore, they also reported an impact of CT on colour intensity, which reached 1.84 at 60 % CT, but only 1.05 in the control. Guidoni *et al.* (2002) showed that CT performed at the pea-size phenological stage on 'Nebbiolo' variety significantly increased soluble solids content by 1 to 2 °Brix. Furthermore, Gil *et al.* (2013) and Prajitna *et al.* (2007) demonstrated that, compared to the control, CT of 'Syrah' and 'Chambourcin' varieties significantly increased anthocyanin contents by 24 % and 52 %, respectively. However, CT could result in a significant yield reduction (Gil *et al.*, 2013; Gamero *et al.*, 2014), although the produced grape quantity usually meets winegrowers expectations. On the other hand, Bubola *et al.* (2011) reported an increased size of the remaining berries after CT. Specifically, berries were 34 % bigger in the 60 % CT treatment compared to the control, but a yield loss of 19 to 40 % was measured. In the last decades, landmark publications regarding the impact of CT on grape and wine quality parameters focused mainly on world-wide cultivated grapevine varieties (Bubola *et al.*, 2011; King *et al.*, 2012; Gil *et al.*, 2013; Šuklje *et al.*, 2013) and less on minor varieties such as 'Blauer Portugieser'. However, local varieties are important for many grape and wine producers, especially when the wine production is targeted and specialized.

'Blauer Portugieser' (*Vitis vinifera* L.) is a red grapevine variety grown in Central and South-Eastern Europe and known for its high yield and vigour (Hrček and Korošec-Koruza, 1996). In Slovenia, the

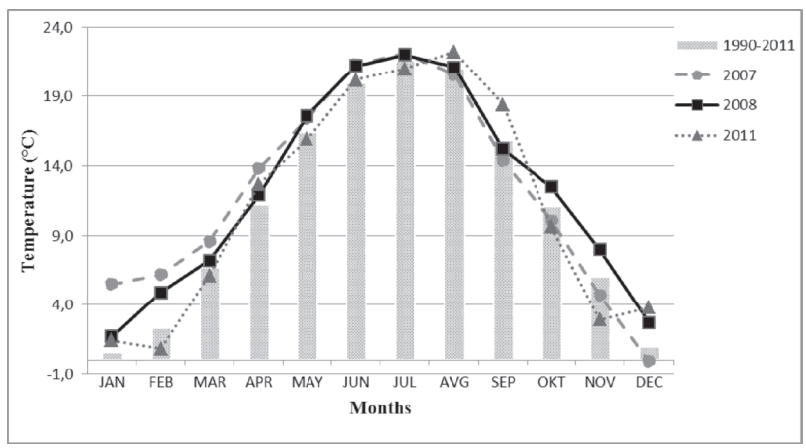

Figure 1 - Average month temperature (°C) of weather station Črnomelj for years 2007, 2008, 2011 and a longterm average between 1990 and 2011.

'Blauer Portugieser' variety (syn. 'Portugalka') is mostly cultivated in the Bela krajina winegrowing district and is one of the most important varieties for the production of a Slovenian wine with a recognised traditional denomination – PTP Metliška črnina (Rules of wine denomination - Metliška črnina, 2008). The high yield of 'Blauer Portugieser' is often reflected in a poor quality (low sugar content, high acidity) and poor colour intensity of grape and wine (Gamero *et al.*, 2014). A long-term monitoring (2002-2012) of grape quality parameters indicated that the 'Blauer Portugieser' variety has, on average, a low sugar content from 67 to 85 °Oe, quite variable total acidity from 4.4 to 7.3 g/L, a pH from 3.14 to 3.62, and an average berry weight from 185 to 268 g/100 berries (Chamber of Agriculture and Forestry of Slovenia, 2014). The Blauer Portugieser wine is known as a light-bodied, light-coloured dry wine with an alcohol content around 11 %, acidity 7 g/L, total extract 25 g/L, pH 3.4 and volatile acidity around 0.2 g/L (Nemanič, 1999). Thus, CT could represent one of the possible practices to improve and standardize grape and wine quality (Šuklje *et al.*, 2013). The aim of the present study was to monitor for the first time the potential impact of different levels of cluster thinning of 'Blauer Portugieser' on yield and growth potential and grape and wine composition.

The research provides, in particular, information about the phenolic profile of 'Blauer Portugieser' which greatly affects grape and wine quality. The results will be useful to winegrowers of 'Blauer Portugieser', who, until now, lacked information on the effect of thinning on grape and wine quality characteristics.

MATERIALS AND METHODS

1. Experimental vineyard

The experiment was conducted in 2007, 2008 and 2011 on 'Blauer Portugieser' vines trained on single Guyot, planted at the distance of 2.3 m x 0.9 m, and grafted on SO4 rootstock (*Vitis berlandieri* × *Vitis riparia*). The vineyard was maintained with a permanent green cover and managed according to integrated pest management measures; it was located in South-Eastern Slovenia in the Bela krajina winegrowing district, in the Radovica winegrowing area (latitude: 45°41' N, longitude: 15°20' E) at 375 m above sea level. The soil was classified as sandy clay loam (> 45 % sand, 20-40 % clay and < 30 % silt), its depth was more than 70 cm, containing 1 to 2 % of organic matter with a medium water-holding capacity. Soil analysis at the depth 0-20 cm showed 5.4 mg P_2O_5/100 g of soil, 21.3 mg K_2O/100 g of soil, 7.8 mg Mg/100 g of soil, 1.6 % organic matter and a pH value of 7.0, and at the depth 20-40 cm 1.5 mg P_2O_5/100 g of soil, 20.1 mg K_2O/100 g of soil, 4.6 mg Mg/100 g of soil, 0.8 % organic matter and a pH value of 7.2. The soil characteristics are typical for anthrosol, which has been formed or heavily modified due to long-term human activity, such as deep ploughing, cultivation or irrigation (Pedological map of Slovenia, 2007). The climate in the area is subpanonic with an average annual air temperature of 10.1 °C and rainfall not exceeding 1100 mm per year, according to the average temperature (Fig. 1) and precipitation (Fig. 2) from 1990 to 2011 (Slovenian Environment Agency, 2015).

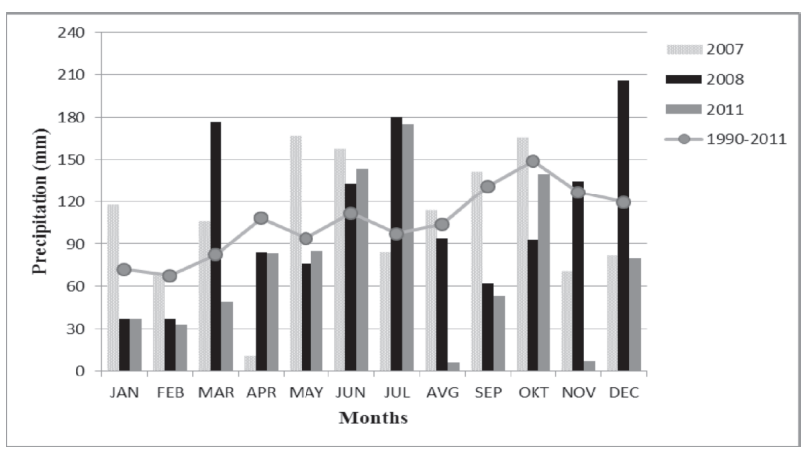

Figure 2 - Average month precipitation (mm) of weather station Črnomelj for years 2007, 2008, 2011 and a longterm average between 1990 and 2011.

2. Experimental design

The study was conducted in a vineyard as a block experiment with three treatments and four replicates per treatment. Each block was built of three randomly set treatments, where each treatment was set on twenty consecutive vines in a single row: control (C - no cluster thinning), cluster thinning 1 (CT1 - all third clusters on the shoot and every second cluster on each second shoot were removed; limited 20-30 % cluster reduction) and cluster thinning 2 (CT2 - only one cluster left on each shoot; severe 40-50 % cluster reduction). The treatment replicates were organized by four parallel and adjacent rows. The initial growth potential was measured - the buds and shoots on studied vines were counted and the yield potential at BBCH 53 (BBCH, 2001) phenological stage was estimated by counting the inflorescences. The level of cluster thinning was set on the basis of recorded yield potential. The cluster thinning was always performed at the pea-size berry phenological stage (BBCH stage 75): in 2007 on 17th of July, in 2008 on 19th of July, and in 2011 on 1st of July. The experiment started in 2007 and was upgraded in 2008 and 2011 with a detailed analysis of secondary metabolites in grape and wine. Approximately 0.5 kg of grapes (4-5 clusters per replicate) were harvested at appropriate ripeness (BBCH 89) and berries were collected from different parts of the cluster for further analyses. Five individual microvinifications per treatment were conducted under equal conditions: 200 kg of grapes per treatment were first divided into five equal parts, the grapes were destemmed, berries crushed and the maceration lasted for 10 days. At the start of the maceration, 20 g/hL of rehydrated yeast *Saccharomyces cerevisiae* Mycoferm ROUGE was added into each vessel, according to the producer guidelines (INRA, Bordeaux, France). During the maceration, the cap of grape marc was punched-down by hand twice per day, and at the end of the maceration process the marc was pressed separately per treatment and replicate. The obtained semi-fermented must was decanted into individual 30-L tanks, where wine fermentation continued for an additional fourteen days. After that, the wine was decanted and 25 mL/hL of 5 % sulphuric acid (H_2SO_3) was added into individual tanks.

3. Measurements and analysis

All measurements of berry characteristics were conducted immediately after harvest, and chemical analyses were carried out during the following months. The growth (vegetative) potential of vines was assessed by counting the total number of unfertile and fertile buds and shoots before flowering in all three years. In the first year (2007) of the study, the weight of 100 berries, contents of soluble solids and titratable acidity were measured; in the next year (2008), the experiment was upgraded with additional measurements of colour parameters of berry skin and must. In the third year (2011), the study focused on secondary metabolites with measurements of the contents of total and individual phenolics in grape and wine. Additionally, colour parameters of berries, chemical parameters of berries and/or wine (pH value, content of soluble solids and titratable acidity) and wine composition parameters were measured.

4. Berry and wine composition

The content of soluble solids was assessed with a digital refractometer (ATAGO PAL87S) and expressed in Brix scale. To determine the content of titratable acidity, 0.1 M NaOH was added to the sample with a semiautomatic titrator till pH value reached 7.0 (Ough and Amerine, 1988a) and the required volume was recorded. The content of titratable acidity was expressed in g/L. Before the titration, initial pH value of the must was measured. The chemical analyses of the produced wines were performed one month after sulphuring. A single sample of wine per tank (five wines/replicate per treatment) was decanted and the chemical characteristics were evaluated using a WineScan FT120 (Foss, Denmark).

5. Colour parameters

Twenty berries per replicate for each individual treatment were randomly sampled and used to determine colour parameters with a Minolta CR-300 Chroma colorimeter (Minolta Co.; Osaka, Japan). Two measurements at opposite sites of the same berry were taken and CIELAB values a*, b*, L*, C* and h were recorded to calculate the CIRG index (Carreño *et al.*, 1995).

The must colour was measured according to the method reported by Ough and Amerine (1988b). The pH value of each must sample was recorded and samples were diluted with a solvent solution of sulphuric acid (H_2SO_4) and bidistilled water of the identical pH value (1:10, v/v). Absorbance was measured on a UV-VIS spectrophotometer (Lambda Zobio) at 420 nm, 520 nm and 620 nm. Colour intensity was calculated as the sum of absorbances A_{420}, A_{520} and A_{620} for each sample and tonality (hue) as the ratio of A_{420} and A_{520} (Glories, 1984).

Table 1 - Identification of phenolic compounds in grape skin and wine of 'Blauer Portugieser' variety in positive and negative ionization with HPLC-MS and MS2.

Tentative identification	M^+ or $[M-H]^-$ (*m/z*)[a]	MS^2 (*m/z*)	Analyzed in	
			skin	wine
Hydroxycinnamic acids				
Caftaric acid	311	179	x	x
p-Coumaroyl pentose	295	163	x	x
trans fertaric acid	325	193	x	x
trans coutaric acid	295	163	x	x
Flavanols				
Catechin	289	245	x	x
Epicatechin	289	245		x
Procyanidin dimer 1	577	289		x
Procyanidin dimer 2	577	289		x
Procyanidin trimer	865	407, 289	x	x
Flavonols				
Myricetin-3-glucoside	479	317	x	x
Myricetin-3-glucuronide	493	317	x	x
Quercetin-3-rutinoside	609	391	x	
Quercetin-3-galactoside	463	301	x	x
Laricitrin-3-glucoside	493	331	x	x
Quercetin-3-glucoside	463	301	x	x
Quercetin-3-glucuronide	477	301	x	x
Laricitrin-3-glucuronide	507	331	x	
Kaempferol-3-*O*-(6-*O*-*p*-coumaroyl) glucoside	593	447, 285	x	
Isorhamnetin-3-rutinoside	623	315	x	x
Kaempferol-3-rutinoside	593	285	x	x
Kaempferol-3-galactoside	447	285	x	x
Kaempferol-3-glucoside	447	285	x	x
Isorhamnetin-3-glucoside	477	315	x	x
Syringetin-3-glucoside	507	345	x	x
Kaempferol-3-glucuronide	461	285	x	
Laricitrin-3-rutinoside	639	331		x
Anthocyanins				
Delphinidin-3-glucoside	465	303	x	
Cyanidin-3-glucoside	449	287	x	
Petunidin-3-glucoside	479	317	x	x
Peonidin-3-glucoside	463	301	x	
Malvidin-3-glucoside	493	331	x	x
Delphinidin-3-(6″ acetyl) glucoside	507	303	x	
Petunidin-3-(6″acetyl) glucoside	521	317	x	
Malvidin-3-(6″acetyl) glucoside	535	331	x	x
Delphinidin-3-(6″-*p*-coumaroyl) glucoside	611	303	x	
Petunidin-3-(6″-*p*-coumaroyl) glucoside	625	317	x	x
Peonidin-3-(6″-*p*-coumaroyl) glucoside	609	301	x	x
Malvidin-3-(6″-*p*-coumaroyl) glucoside	639	331	x	x
Malvidin-3-glucoside acetate	517	355		x
Malvidin-3-glucoside pyruvate	561	399		x
Malvidin-3-(6″acetyl) glucoside pyruvate	603	399		x
Peonidin-3-(6″acetyl) glucoside	505	301		x
Malvidin-3-(6″caffeoyl) glucoside	655	331		x
Malvidin-3-(6″-*p*-coumaroyl) glucoside pyr	707	399		x

[a] M^+ (m/z) anthocyanins were obtained in positive ion mode, other phenolics in negative ion mode.

6. Phenolic compounds

Phenolic compounds were extracted from berry skin according to the method of Mikulič-Petkovšek *et al.* (2012). Briefly, the skin was separated from the pulp with a scalpel when the berries were still frozen, and the skin samples were ground to a fine powder with liquid nitrogen. Approximately 0.5 g of the skin powder (exact weight was recorded for each sample) was put into a 10-mL centrifuge tube, and 10 mL of methanol containing 3 % (v/v) formic acid and 1 % (w/v) 2,6-di-tert-butyl-4-methylphenol (BHT) were added. BHT was added to the samples to prevent oxidation. The extraction was performed in a cooled ultrasonic bath for 1 h. After extraction, the obtained extracts were centrifuged (Eppendorf Centrifuge 5810R) at 10.000 rpm for 7 min at 4 °C. The supernatant was filtered through a 0.20-µm injection filter (Chromafil A-20/25, Macherey-Nagel) into a vial. Phenolic analyses were performed on a HPLC-MS system (Thermo Finnigan Surveyor with quaternary pump, San Jose, USA); a Phenomenex Gemini C18 (150 mm x 4.5 mm, 3 µm) column operating at 25 °C was used. Phenolics were detected at 280 nm (flavanols, hydroxycinnamic acids derivatives), 350 nm (flavonols) and 530 nm (anthocyanins). The elution solvents were aqueous 1 % formic acid (A) and 100 % acetonitrile (B). Samples were eluted according to the linear gradient described by Marks *et al*. (2007), with an injection amount of 20 µL and flow rate of 1 mL/min. All phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in negative (all phenolic groups except anthocyanins) and positive (anthocyanins) ion mode. The analyses were carried out using full scan data-dependent MS^n scanning from *m/z* 115 to 1500. The injection volume was 10 µL and the flow rate maintained at 1 mL/min. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, respectively, the source voltage was 3 kV for negative ionization and 4 kV for positive ionization, and normalized collision energy was between 20-35 %. Spectral data were elaborated using the Excalibur software (Thermo Scientific).

The identification of phenolic compounds was confirmed by comparing retention times and their spectra as well as by adding the standard solution to the sample and by fragmentation (Table 1). The content of total hydroxycinnamic acids, flavanols, flavonols and anthocyanins was calculated as the sum of all identified and quantified individual phenolics of the corresponding phenolic group.

7. Total phenolic content (TPC)

Total phenolic content was assessed with the Folin-Ciocalteu reagent method described by Singleton *et al.* (1999). The extraction of phenolics from berry skin was made according to the protocol reported by Mikulič-Petkovšek *et al.* (2012), but without adding the BHT into the extraction solution to avoid interferences with phenolics that may indicate an increase of the analytical value. 100 µL of the extract was placed into a centrifuge tube and 6 ml of bidistilled water and 500 µL of Folin-Ciocalteu reagent were added to the mixture. After 1 to 8 minutes, 1.5 mL of sodium carbonate (Na_2CO_3) was added and the mixture was diluted with bidistilled water until reaching a volume of 10 ml. The samples were mixed and heated in a drying oven at 40 °C for 30 min. The samples were poured into 1.5-mL cuvettes and the absorbance was measured on a spectrophotometer (Perkin-Elmer, UV/visible Lambda Bio 20) at 765 nm. Water and corresponding reagents were used as a blank. Different concentrations of gallic acid were measured to obtain an absorbance standard curve. The absorbance of each sample was measured in three replicates. TPC was expressed as gallic acid equivalents (GAE) in mg/kg FW of berry skin/wine in mg/L.

8. Chemicals

The following standards were used for the quantification of phenolic compounds: cyanidin-3-*O*-galactoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, caffeic acid, catechin, *p*-coumaric acid and procyanidin B2 from Fluka Chemie GmBH (Buchs, Switzerland), delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, quercetin-3-*O*-rutinoside and kaempferol-3-glucoside from Sigma-Aldrich Chemical (St. Louis, MO, USA). To prevent the oxidation of individual phenolics, 1 % (w/v) BHT from Sigma-Aldrich GmBH was used. The Folin-Ciocalteu reagent from Fluka Chemie GmBH (Buchs, Switzerland) was used to determine TPC. Methanol for extraction of phenolics was from Sigma-Aldrich GmBH. The chemicals used for the mobile phases were acetonitrile HPLC-MS grade and formic acid from Fluka Chemie GmBH. Water was purified and twice distilled with a Milli-Q-system (Millipore, Bedford, MA, USA).

9. Statistical analysis

Data were analyzed using the Statgraphics Centurion XV program (Statpoint Technologies Inc., Warrenton, Virginia, USA). Differences in yield, grape and wine composition among treatments were assessed with one-way analysis of variance

Table 2 - Growth potential and yield characteristics of 'Blauer Portugieser' grapevines at different cluster thinning levels in three years

Growth potential						
Treatment	Buds			Shoots		
2007	Total	Unfertile	Fertile	Total	Unfertile	Fertile
C	13.3±0.4	1.0±0.2	12.9±0.7	13.6±0.6	0.3±0.1	13.3±0.5
CT1	14.0±0.5	1.3±0.3	13.5±0.5	14.3±0.5	0.6±0.2	13.7±0.5
CT2	14.4±0.4	1.4±0.2	12.9±0.3	13.8±0.4	0.3±0.1	13.4±0.4
2008						
C	14.0±0.4	0.8±0.3	12.7±0.5	15.4±0.8	0.3±0.1	14.8±0.7
CT1	13.8±0.7	0.7±0.3	12.4±0.7	14.6±0.8	0.7±0.2	13.7±0.8
CT2	13.7±0.3	1.0±0.3	12.4±0.5	13.7±0.6	0.8±0.2	12.9±0.6
2011						
C	12.8±0.6	1.1±0.3	11.4±0.7	11.2±0.4	0.5±0.3	10.7±0.4
CT1	12.3±0.4	1.5±0.3	11.1±0.5	10.4±0.7	0.5±0.2	9.9±0.7
CT2	13.3±0.4	1.3±0.3	11.4±0.5	10.6±0.5	0.1±0.1	10.7±0.5

Yield characteristics						
Treatment	No. of clusters per vine at flowering	No. of removed clusters per vine	No. of clusters per vine at harvest	Yield (kg/vine)	Cluster weight (g)	Yield potential (t/ha)
2007						
C	24.3±1.0	0±0 a	23.8±1.0 c	3.0±0.2 b	127±7.9	14.3±0.9 b
CT1	24.4±0.9	5.5±0.6 b	19.9±0.9 b	2.8±0.3 b	142±11.5	13.5±1.4 b
CT2	24.8±1.0	10.3±0.7 c	15.4±0.7 a	2.1±0.2 a	135±9.2	10.1±1.0 a
2008						
C	24.8±1.2	0±0 a	24.2±1.1 c	3.8±0.2 b	161±8.1	18.1±0.7 b
CT1	23.7±0.9	5.6±0.8 b	18.3±0.8 b	3.3±0.2 ab	183±12.2	15.7±0.9 ab
CT2	22.0±0.9	9.6±0.9 c	14.0±0.8 a	2.7±0.3 a	194±15.5	13.0±1.3 a
2011						
C	18.8±0.6	0±0 a	18.3±1.1 b	2.6±0.2 b	142±7.6	12.3±1.0 b
CT1	16.1±1.0	5.3±0.7 b	11.3±1.2 a	1.8±0.1 a	133±9.0	7.7±1.4 a
CT2	18.3±1.6	8.3±0.9 c	11.0±0.9 a	1.5±0.2 a	126±14.1	7.0±0.7 a

C : control (no cluster thinning) ; CT1 : 20-30 % cluster reduction ; CT2 : 40-50 % cluster reduction. The results are presented as mean ± standard error and different letters in columns denote statistically significant differences (LSD test, $p < 0.05$) among treatments in each individual year.

(ANOVA). Significant treatment means were determined using Fisher's least significant difference (LSD) and Duncan multiple range test at 95 % confidence level ($p \leq 0.05$), and significant differences are given by different letters.

RESULTS

1. Growth and yield potential

No significant differences in growth potential were observed between the different levels of CT in a single year, confirming equal growing conditions in the vineyard (Table 2). Irrespective of the year, an average of 12.3 to 14.4 buds/vine were left at winter pruning, which resulted in an average of 10.4 to 15.4 shoots/vine, of which 0.1 to 0.8 were unfertile. No significant differences in cluster number/vine were observed among treatments at flowering, which reached 16.1 to 24.8 clusters/vine, irrespective of the year. However, the reduction of clusters (CT1 and CT2) was made according to the recorded total number of clusters per individual vine. All clusters were left on the vines in the control treatment and, in accordance with the experimental design, the number of removed clusters was significantly higher in the CT2 treatment (8.3 to 10.3 clusters/vine) compared to the CT1 treatment (5.3 to 5.6 clusters/vine), irrespective of the year. As expected, different levels of CT resulted in significantly different yields/vine, especially in CT2 compared to the control. Taking into account all three years, vines subjected to the

Table 3 - Characteristics of 'Blauer Portugieser' grape and wine composition at different cluster thinning levels in three years

Parameters	2007			2008			2011		
	C	CT1	CT2	C	CT1	CT2	C	CT1	CT2
Grape									
Soluble solids (°Brix)	17.2±0.4 a	18.5±0.7 ab	18.6±0.4 b	15.4±0.3 a	16.3±0.3 a	18.3±0.8 b	18.8±0.3 a	19.1±0.9 a	21.5±0.3 b
Titratable acidity (g/L)	5.3±0.05 b	4.2±0.13 a	3.7±0.08 a	4.2±0.05 b	3.5±0.04 a	3.6±0.01 a	4.2±0.06 b	4.1±0.01 b	3.3±0.04 a
pH	3.4±0.04 a	3.8±0.02 b	3.8±0.02 b	3.3±0.04 a	3.7±0.03 b	3.8±0.05 b	3.2±0.04 a	3.7±0.03 b	3.8±0.03 b
Weight of 100 berries (	165±6.5	172±4.8	168±6.2	161±6.9	170±13.0	163±7.5	170±7.7	176±4.4	174±5.2
CIRG index	-	-	-	6.3±0.2 a	6.9±0.1 b	7.0±0.2 b	6.1±0.2 a	7.2±0.3 b	7.2±0.2 b
Colour Intensity	-	-	-	5.7±0.1 a	6.7±0.1 b	8.7±0.3 c	8.8±0.1 a	10.8±0.9 b	11.6±0.3 b
Colour Tonality (Hue)	-	-	-	1.1±0.1 a	1.3±0.1 b	1.3±0.3 ab	1.3±0.1	1.3±0.1	1.3±0.1
Wine									
Alcohol (vol.%)	-	-	-	-	-	-	10.3±0.1 a	11.5±0.2 b	11.8±0.1 b
Total acidity (g/L)	-	-	-	-	-	-	6.9±0.05 c	6.4±0.03 b	6.2±0.01 a
pH	-	-	-	-	-	-	3.56±0.01 a	3.56±0.02 a	3.60±0.01 b
Total extract (g/L)	-	-	-	-	-	-	27.6±0.2 a	28.3±0.1 ab	29.0±0.2 b
Volatile acidity (g/L)	-	-	-	-	-	-	0.28±0.01 a	0.31±0.01 b	0.32±0.02 b

″-″not analyzed; C: control; CT1: 20-30 % cluster reduction; CT2: 40-50 % cluster reduction. The results are presented as mean ± standard error and different letters in rows denote statistically significant differences (LSD test, $p < 0.05$) among treatments in each individual year.

CT2 treatment produced 1.5 to 2.7 kg of grapes, but no significant reduction in yield/vine was observed when compared to CT1 in 2008 and 2011 (Table 2). CT did not significantly affect average cluster weight, which ranged from 126 to 194 g. The results indicate that limited cluster removal (CT1) in 'Blauer Portugieser' variety does not generally reduce yield/ha when compared to the control. This was confirmed in 2007 and 2008. On the other hand, severe cluster removal (CT2) significantly decreased yield/ha compared to the control treatment in all three years of the study by an average of 4.2 to 5.3 t/ha. However, taking into account the average cluster weight, yield per vine and number of vines/ha, the yield/ha in CT2 was quite high, reaching 7.0 to 13.0 t/ha, also in 2011 when the number of buds/vine at winter pruning was further reduced.

2. Grape and wine composition

CT affected many of the studied grape and wine parameters (Table 3). Only the most severe CT (CT2) significantly increased the average content of soluble solids by 1.4 to 2.9 °Brix in comparison to the control, whereas the average content of soluble solids in CT1 was not significantly higher compared to the control in all three years. A greater impact of CT was observed on the content of titratable acidity (TA) and pH value: irrespective of the year, TA significantly decreased, while pH significantly increased (Table 3). Although the average berry weight was not affected by CT, the berries from CT vines reached significantly higher CIRG index and colour intensity, but not tonality, in 2011. In 2011, wine composition was additionally studied. Compared to the control, CT significantly increased the alcohol content by 1.2 to 1.5 vol. % and volatile acidity (which reached 0.31 and 0.32 g/L in CT1 and CT2, respectively), but decreased total acidity by 0.5 to 0.7 g/L. Moreover, CT2 significantly increased wine pH and the content of total extract.

3. Phenolic composition of grape skin

Thirty-four different phenolic compounds were identified and quantified in 'Blauer Portugieser' berry skin (Table 4a and b). Flavonols (16 compounds) were the most important group of identified phenolics in grape skin, followed by anthocyanins (12), hydroxycinnamic acids (4) (HCA) and flavanols (2). Generally, CT2 significantly increased the content of most phenolic compounds. Only CT2 significantly increased the content of total hydroxycinnamic acids (THCA) by an average of 16 mg/kg compared to the control. Caftaric acid was the most abundant among HCA. Its content was significantly increased by CT2 compared to the control, but its relative abundance was not affected. CT2 significantly increased the content and the proportion of *p*-coumaroyl pentose, which reached 12.8 mg/kg and its proportion was 1.2 % higher than in control. CT did not affect the content of total flavanols (TFA), but the proportion of catechin and procyanidin trimer significantly

Table 4a - Concentration of phenolic compounds in berry skin (mg/kg) and wine (mg/L) and relative abundance (%) according to the total amount of each phenolic group of 'Blauer Portugieser' subjected to different cluster thinnings

Phenolic compounds	Treatment													
	Skin (mg/kg)							Wine (mg/L)						
	C	%	CT1	%	CT2	%	PI	C	%	CT1	%	CT2	%	PI
Caftaric acid	105±2.8 a	82	114±5.1 ab	81	122±3.2 b	82	↑**	9.7±0.2 a	56	8.7±0.9 a	54	11.8±0.2 b	55	↑**
p-cou pentose	9.7±0.4 a	6:00 AM	8.9±0.6 a	7:00 AM	12.8±1.1 b	9 b	↑**	3.5±0.7 a	19 a	3.3±1.1 a	20 a	5.4±0.3 b	25 b	↑**
t-fertaric acid	11.2±5.6	8	10.4±5.0	8	7.5±4.0	5		2.7±0.5	15	2.9±1.0	18	3.0±0.4	14	
t-coutaric acid	6.4±0.2 b	4	5.4±0.3 a	4	5.6±0.3 ab	4		1.7±0.1	10 b	1.3±0.4	8 ab	1.4±0.1	6:00 AM	
Total hydroxycinnamic acids (THCA)	**132±5.2 a**	**100**	**139±4.6 a**	**100**	**148±3.3 b**	**100**	↑**	**17.5±1.5 a**	**100**	**16.1±5.4 a**	**100**	**21.5±0.4 b**	**100**	↑**
Catechin	167±3.3 a	40 a	143±9.5 a	46 b	209±6.2 b	48 b	↑**	39.0±1.2	16	39.1±1.3	15	51.4±3.7	14	
Procyanidin dimer 1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		29.3±3.7 a	12:00 AM	31.0±1.2 a	12:00 AM	55.4±2.7 b	15 b	↑**
Procyanidin dimer 2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		112±4.6 a	45 b	113±4.4 a	43 ab	146±2.4 b	40 a	↑**
Procyanidin trimer	254±15.5	60 b	191±48.3	54 a	271±82.8	52 a		26.9±2.2 a	11	27.1±9.1 a	11	38.1±2.5 b	11	↑**
Epicatechin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		41.1±1.3 a	17 a	48.7±1.8 a	19 ab	74.1±5.4 b	20 b	↑**
Total flavanols (TFA)	**421±15.5**	**100**	**334±57.0**	**100**	**480±78.1**	**100**		**248±10.7**	**100**	**259±92.9**	**100**	**365±18.9**	**100**	
Myricetin-3-glu	90.6±8.4 a	10:00 AM	82.2±11.1 a	13 ab	124±8.4 b	16 b	↑**	4.2±0.3 a	8:00 AM	5.1±0.2 ab	9 b	7.9±0.1 b	10 c	↑**
Myricetin-3-gluc	22.7±1.4 a	3	29.3±2.1 b	3	31.0±0.5 b	3	↑*	0.6±0.2	1	0.6±0.2	1	0.7±0.1	1	
Quercetin-3-rut	25.9±2.9 a	4	26.8±1.8 b	4	35.0±1.5 b	3	↑*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Quercetin-3-gal	46.4±5.4	6	55.5±1.4	6	65.2±10.0	7		4.1±0.1	8:00 AM	4.9±1.7	9 b	6.7±0.2	9 b	
Laricitrin-3-glu	4.6±0.2	0.5	4.1±0.5	0.6	5.6±0.7	1		4.2±0.1 a	13 a	5.1±1.7 ab	17 b	7.6±0.1 b	17 b	↑**
Quercetin-3-glu	137±4.7	15 a	123±15.9	17 ab	167±21.7	18 b		1.6±0.2 a	4:00 AM	2.7±0.9 ab	5 b	3.9±0.3 b	5 b	↑**
Quercetin-3-gluc	283±20.6 a	43 b	327±33.7 ab	40 b	397±16.2 b	36 a	↑**	21.8±0.2 a	44 b	20.0±0.7 a	37 a	29.5±0.1 b	38 a	↑**
Laricitrin-3-gluc	2.8±0.2 a	0.4	3.3±0.3 ab	0.4	3.9±0.2 b	0.4	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Kaempferol-3-(6-*p*-cou)	25.0±2.0 a	4	27.9±1.7 b	3	35.0±1.1 b	3	↑*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Isorhamnetin-3-rut	35.3±1.8	4	25.2±2.2	4	61.5±38.1	6		2.6±0.2	5	1.7±0.8	12	2.9±0.4	4	
Kaempferol-3-rut	2.9±0.5	0.4	2.9±0.4	0.4	3.9±0.1	0.3		0.1±0.01	0.2	0.1±0.04	0.2	0.2±0.02	0.2	
Kaempferol-3-gal	17.0±0.5	2	12.4±2.0	2	12.5±1.6	1		0.9±0.1	2	1.1±0.3	2	1.7±0.2	2	
Kaempferol-3-glu	50.6±8.3	6	57.7±3.7	7	61.5±3.4	6		0.03±0.01	0.1	0.04±0.02	0.1	0.06±0.01	0.1	
Isorhamnetin-3-glu	2.9±0.1	0.3	3.4±0.3	0.5	3.6±0.9	0.4		6.7±0.1	13 c	6.7±2.2	12 b	8.9±0.2	11:00 AM	
Syringetin-3-glu	1.8±0.1	0.2	2.2±0.2	0.3	2.4±0.6	0.3		0.9±0.01	2	0.9±0.03	2	1.3±0.02	2	
Kaempferol-3-gluc	5.1±0.2	0.6 a	5.9±0.5	0.8 a	6.4±1.6	1 b		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Laricitrin-3-rut	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		1.0±0.1	2	1.4±0.5	3	1.8±0.1	2	
Total flavonols (TFO)	**755±18.7 a**	**100**	**789±16.9 a**	**100**	**1017±19.3 b**	**100**	↑**	**50.0±0.6 a**	**100**	**54.2±1.1 a**	**100**	**77.9±1.5 b**	**100**	↑**

Table 4b - Concentration of phenolic compounds in berry skin (mg/kg) and wine (mg/L) and relative abundance (%) according to the total amount of each phenolic group of 'Blauer Portugieser' subjected to different cluster thinnings

Phenolic compounds	Treatment													
	Skin (mg/kg)							Wine (mg/L)						
	C	%	CT1	%	CT2	%	PI	C	%	CT1	%	CT2	%	PI
Delphinidin-3-glu	588±7.4 b	7:00 AM	505±6.9 a	7:00 AM	814±25.1 c	12 b	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Cyanidin-3-glu	733±21.0 a	11	660±32.5 a	10	848±22.1 b	10	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Petunidin-3-glu	718±15.03 a	9:00 AM	628±25.7 a	10:00 AM	906±57.7 b	13 b	↑**	9.2±0.2 a	3	14.7±0.9 b	4	13.9±0.2 b	4	↑*
Peonidin-3-glu	459±22.8 a	6:00 AM	405±34.2 a	7:00 AM	553±12.9 b	8 b	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Malvidin-3-glu	1765±41 a	29 b	2105±44 b	30 b	2244±76 b	27 a	↑*	182±1.7	65 c	156±5.2	64 b	199±3.3	62 a	
Malvidin-3-glu ac	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		1.1±0.1 a	0.5 a	1.4±0.2 b	0.4 a	1.9±0.3 c	1 b	↑*
Malvidin-3-glu py	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		22.3±0.6 a	8:00 AM	30.0±0.2 b	9 b	28.8±0.3 b	9 b	↑*
Malvidin-3-(6″acetyl) glu	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		7.4±0.2 a	9	9.5±0.4 b	8	9.9±0.1 b	9	↑*
Peonidin-3-(6″acetyl) glu	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		4.1±0.2	1	4.5±0.1	1	4.5±0.1	1	
Malvidin-3-(6″caffeoyl) g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		2.4±0.3 a	1	2.7±0.1 b	1	2.9±0.5 c	1	↑*
Malvidin-3-(6″-*p*-cou) gl	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		4.1±0.1 a	1:00 AM	5.1±0.2 b	2 b	5.7±0.1 c	2 b	↑*
Delphinidin-3-(6″ acetyl)	53.6±1.4 a	0.7	46.6±2.5 a	0.8	91.7±5.5 b	1	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Petunidin-3-(6″acetyl) glu	219±5.4 b	3	187±1.3 a	3	243±1.2 c	3	↑**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Malvidin-3-(6″acetyl) glu	299±18.9 a	6	318±28.4 a	5	442±15.8 b	5	↑*	25.2±0.4 a	9 b	26.8±0.1 ab	8:00 AM	28.3±0.6 b	8:00 AM	↑**
Delphinidin-3-(6″-*p*-cou)	186.9±5.8 a	3	175±12.4 a	3	232±7.7 b	3	**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Petunidin-3-(6″-*p*-cou) gl	273±14.1 a	4	261±15.8 a	4	368±11.9 b	4	↑**	3.1±0.4 a	1	3.5±0.1 b	1	3.9±0.2 c	1	↑*
Peonidin-3-(6″-*p*-cou) glu	307±33.9 a	5	290±21.2 a	5	413±30.1 b	5	↑**	3.3±0.1 a	1	4.2±0.3 b	1	4.5±0.6 c	1	↑*
Malvidin-3-(6″-*p*-cou) gl	729±47.9 a	14 c	791±73.0 a	12 b	1140±53.0 b	########	↑*	13.8±0.4 a	1:00 AM	18.01±0.4 b	5 ab	19.9±0.1 c	6 b	↑*
Total anthocyanins (TA	**6334±75 a**	**100**	**6376±68 a**	**100**	**8299±196 b**	**100**	↑**	**279±3.1 a**	**100**	**328±1.9 b**	**100**	**323±4.1 b**	**100**	↑*
TPC	**8069±157 a**		**8102±127 a**		**9339±159 b**		↑**	**880±17.3 a**		**1045±28.8 b**		**1075±11.3 b**		↑*

Abbreviations of phenolic names: t: *trans*, *p*: para, glu: glucoside, gluc: glucuronide, rut: rutinoside, cou: coumaroyl, ac: acetate, py: pyruvate.
N.D. – not detected; C: control (no cluster thinning); CT1: 20-30 % cluster reduction; CT2: 40-50 % cluster reduction. TPC – total phenolic content (mg GAE/kg or mg GAE/L). PI – potential impact: ↑* or ↓* - potential increase or decrease among control and thinning treatments. ↑** or ↓** - potential increase or decrease in CT2 compared to C or CT1. The results are presented as mean ± standard error and as proportion (%) of each phenolic compound according to total value of associated phenolic group within individual treatment. Different letters in rows denote statistically significant differences (LSD test, $p < 0.05$) among treatments.

changed after CT. CT significantly increased the accumulation of less than half of the identified flavonols in berry skin. However, the content of total flavonols (TFO) increased after CT2, reaching 1017 mg/kg of fresh weight (Table 4). The most abundant flavonols in 'Blauer Portugieser' berry skin, irrespective of the treatment, were quercetin-3-glucuronide, quercetin-3-glucoside, myricetin-3-glucoside and quercetin-3-galactoside. CT2 only significantly increased quercetin-3-glucuronide and myricetin-3-glucuronide levels in comparison to the control. Interestingly, CT2 significantly increased the content of quercetin-3-glucuronide, but decreased its proportion regarding the total flavonol (TFO) content. Furthermore, a significant increase in the proportion of myricetin-3-glucoside, quercetin-3-glucoside and kaempferol-3-glucuronide was observed following severe CT. In agreement with the results obtained for flavonols, the most severe cluster thinning (CT2) had a significant impact on the total anthocyanin content (TAC) and on most of the identified individual anthocyanins in berry skin (Table 4). The most abundant of them was malvidin-3-glucoside, representing 27 to 30 % of TAC, followed by malvidin-3-(6″-*p*-coumaroyl) glucoside (10-14 % TAC), petunidin-3-glucoside (9-13 % TAC), cyanidin-3-glucoside (10-11 % TAC), delphinidin-3-glucoside (7-12 % TAC) and peonidin-3-glucoside (6-8 % TAC). CT2 significantly increased the content and proportion of delphinidin-, cyanidin-, petunidin-, and peonidin-3-glucoside, compared to the control. However, the content of malvidin-3-glucoside and malvidin-3-(6″-*p*-coumaroyl) glucoside also significantly increased in CT2, but the treatment decreased their proportion regarding TAC. Furthermore, TPC in CT1 vines was comparable to the control vines, suggesting that only a severe cluster reduction (CT2) may affect the accumulation of phenolics in berry skin of 'Blauer Portugieser'.

4. Phenolic composition of wine

Phenolic compounds were assessed in wines of the 2011 vintage (Table 4). CT significantly increased the content of several individual phenolic compounds in all four identified phenolic groups in comparison to the control. Caftaric acid was the major hydroxycinnamic acid (8.7 to 11.8 mg/L) identified in wine, followed by *p*-coumaroyl pentose (3.3 to 5.4 mg/L); the content of both significantly increased in CT2 vines, compared to the control (Table 4). On the other hand, the proportion of caftaric acid, regarding THCA, remained stable also in CT2, whereas that of *p*-coumaroyl pentose significantly increased by 6 % and *t*-coutaric acid decreased by 4 %.

Flavanols were the second most prevalent phenolic group in Blauer Portugieser wine, representing almost 43 % of analyzed phenolics. The major flavanol identified in wine was procyanidin dimer 2 (112 to 146 mg/L), representing 40 to 45 % of total flavanols, irrespective of the treatment (Table 4). Among flavanols, only procyanidin dimer 1 and epicatechin responded to CT, and their content and proportion significantly increased only in CT2 compared to the control. The content of procyanidin dimer 2 and procyanidin trimer was similarly increased in CT2 ; however, the proportion of procyanidin trimer remained stable and that of procyanidin dimer 2 significantly decreased.

In total, thirteen flavonols were identified and quantified in Blauer Portugieser wine, the most abundant being quercetin-3-glucuronide, laricitrin-3-glucoside, isorhamnetin-3-glucoside and myricetin-3-glucuronide. Only CT2 had a positive impact on the content of myricetin-3-glucoside, laricitrin-3-glucoside, quercetin-3-glucoside and quercetin-3-glucuronide, with CT1 showing less marked increases, and their proportion significantly increased in comparison with the control, with the only exception of quercetin-3-glucuronide, which decreased significantly after CT.

In addition, CT had a great impact on the content of individual and total anthocyanins (TAC) in wine (Table 4). TAC was significantly higher in both CT treatments compared to the control. The composition of individual phenolics in berry skin was slightly different to that of wine ; five additional malvidin glycosides and peonidin-3-(6″acetyl) glucoside were identified in Blauer Portugieser wine. On the contrary, delphinidin- and cyanidin-glycosides were not detected in wine. Malvidin-3-glucoside was the main anthocyanin, ranging from 156 to 199 mg/L and representing 62 to 65 % of TAC. Although CT did not affect the content of malvidin-3-glucoside, its relative proportion significantly decreased in CT vines. TPC in Blauer Portugieser wine was also affected by CT, with a significant increase in CT1 (1045 mg/L) and CT2 (1075 mg/L) treatments compared to the control wine (880 mg/L).

DISCUSSION

1. Growth and yield potential

'Blauer Portugieser' is a vigorous variety and Reynolds *et al.* (2004) recommend a careful control of the number of buds and clusters per vine at winter pruning and in the growing season to ensure optimal yield and quality of grapes and wine. Growth and yield potential also depend on climatic conditions

(temperature, precipitation, and sunshine hours), which delay or alter phenological stages, shorten their duration or interrupt differentiation (Jones and Davis, 2000). Bubola *et al.* (2011) reported that a smaller number of clusters left on the vine resulted in 18-57 % greater bunch weight of the remaining grapes of the 'Merlot' variety. This was not confirmed for our variety. Moreover, the same authors reported a 34 % decrease in average yield after cluster thinning. A similar pattern was observed in our study: yield decreased significantly by 27-43 % in CT2, especially in 2011 when the vines formed less buds and clusters. Calonnec *et al.* (2004) reported that pests and diseases greatly reduce the yield, but no pathogenic organisms have been recorded in the experimental vineyard. Therefore, the yield decrease in 2011 could also be due to lower average temperatures (21 °C) and above-average precipitation in July (approximately 175 mm), followed by extremely high temperatures and low rainfall (approximately 5 mm) in August (Fig. 1 and Fig. 2), which can cause economic losses (Jackson and Lombard, 1993; Gil *et al.*, 2013). According to the rules of wine denomination - Metliška črnina (2008), the maximum yield of 'Blauer Portugieser' is limited to 11 t/ha as a five-year average yield, which seemed to be achieved only in the severe CT treatment (40-50 % cluster reduction).

2. Grape composition

Cluster thinning generally involves a considerable reduction in yield which potentially improves grape and wine quality and composition (Dokoozlian and Hirschfelt, 1995; Di Profio *et al.*, 2011). Soluble solids content, titratable acidity and pH value still represent the main and basic parameters determining the optimal harvest time (Prajitna *et al.*, 2007), which can be affected by climatic conditions in the growing season (Jones and Davis, 2000). Higher temperatures and precipitation during the ripening process (July and August) in our study might have affected soluble solids content and titratable acidity, especially in 2008 and 2011, when precipitation was above average in July (around 180 mm), compared to the long-term average (approximately 95 mm) (Fig. 1 and Fig. 2). Soil type can also impact vine development, soluble solids content, acidity and pH (Koundouras *et al.*, 2006). Clay soil increases sugar accumulation and speeds the ripening process in comparison to gravel or sandy soil and may influence fruit ripening through mineral supply and water-holding capacity (Jackson and Lombard, 1993). A severe P_20_5 deficit has been observed in the experimental vineyard (Leskošek, 1993) but Jackson and Lombard (1993) could not determine any direct relation between soil mineral supply and grape quality parameters. In general, CT affected grape composition of 'Blauer Portugieser', where soluble solids were generally higher in CT treatments in comparison to the control. On the contrary, titratable acidity in grape significantly decreased as a result of cluster reduction in two years of the study, which is in accordance with the report by Di Profio *et al.* (2011). Jackson and Lombard (1993) reported that the pH value of berry juice impacts the suitability and fermentation conditions of must, which was expected also in our study with CT, where significantly higher pH values were measured, compared to the control. Prajitna *et al*. (2007) showed an insignificant impact of cluster thinning on pH value, but it is known that a lower content of titratable acidity also results in higher pH value (Reynolds, 1989), which is in accordance with our results. Titratable acidity of 'Blauer Portugieser' grapes was much lower in all years/treatments compared to data reported by Pavloušek and Kumšta (2011). This could be explained by different climatic conditions, especially higher temperatures, which lead to acid degradation (Jackson and Lombard, 1993) or by the use of a different clone (Pavloušek and Kumšta, 2011). Berry skin colour is an important characteristic, especially for red grapevine varieties. It is directly affected by composition and content of phenolic compounds (Mazza *et al.*, 1999) and is usually represented by colour intensity, tonality (hue) and CIRG index (Carreño *et al.*, 1995). Colour intensity and tonality (hue) mostly depend on the variety and agronomical practices (Di Profio *et al.*, 2011), cluster thinning (Ough and Nagaoka, 1984; Guidoni *et al.*, 2002), water stress and terroir (Jones and Davis, 2000). The colour intensity of 'Blauer Portugieser' in the CT2 treatment reached values higher than that of 'Sangiovese' (Parpinello *et al.*, 2015) and 'Tempranillo' (Gamero *et al.*, 2014).

3. Wine composition

Many factors affect wine composition, the most important is certainly grape quality at harvest (Gil *et al*., 2013). Nowadays, the demand for less acidic, extract-rich wines is rising (Parpinello *et al.*, 2015). Blauer Portugieser wine is characterized by an intense dark red colour and lower acidity and is mostly drunk young (Nemanič, 1999). On the other hand, a relatively high proportion of 'Blauer Portugieser' grape harvested in Slovenia is used for the production of PTP Metliška črnina (Rules of wine denomination - PTP Metliška črnina, 2008).

Although the Blauer Portugieser wine was produced only in 2011, we observed some interesting and

significant differences in wine composition which could be attributed to CT, especially the content of alcohol, total acidity, total extract and volatile acidity. Moreover, alcohol content in CT2 exceeded the limit defined by the rules (11.5 vol. %), but the difference in alcohol content between the control and CT2 was just 1.5 vol. %. However, wine from the CT2 treatment still meets the current preferences for wines with lower alcohol content (Parpinello *et al.*, 2015). Total acidity seemed to be significantly reduced by thinning, which is in accord with the report of Bubola *et al*. (2011) for Merlot wine. The Blauer Portugieser wine is known as less acidic (Nemanič, 1999; Shellie, 2007), a trait not observed in our study, where 6.2 g/L of total acidity was measured in the severe CT2 wine. However, all analyzed wine samples met the limits set for PTP Metliška črnina (5.5-7 g/L). Contents of total extract and volatile acidity significantly increased in CT2 and met the limits set for PTP Metliška črnina, where a maximum of 0.8 g/L of volatile acidity is permitted. The study suggests that CT, especially with 40-50 % cluster removal, has a remarkable impact on wine composition.

4. Phenolic composition of grape berries and wine

Phenolics are important bioactive compounds in berries and wine, and they play a great role in sensorial characteristics such as berry colour, aroma, flavour, taste and nutritional value (Rodríguez-Montealegre *et al.*, 2006; Castillo-Muñoz *et al.*, 2007). Moreover, these compounds are involved in preventing oxidation and stabilizing the colour of wine (Gómez-Plaza *et al.*, 2000). Phenolic composition and contents are influenced by variety and vineyard practices (Mazza *et al.*, 1999), plant status (Rusjan *et al.*, 2012) and pedoclimatic conditions during ripening (Koundouras *et al.*, 2006). In the present study, higher temperatures in August and September (Fig. 1) and low precipitation in August 2011 (Fig. 2) could have caused stress conditions to the vines, which usually result in increased phenolic accumulation (Koundouras *et al.*, 2006).

Among the HCA group, caftaric acid was the most abundant in 'Blauer Portugieser' berry skin, followed by *p*-coumaroyl pentose. This is not in accordance with the report by Rodríguez-Montealegre *et al.* (2006), who found *t*-coutaric acid as the main HCA in several other red grapevine varieties. In general, higher contents of some individual and total HCA have been measured in 'Blauer Portugieser' berry skin compared to 'Cabernet Sauvignon', 'Merlot' and 'Shiraz' (Rodríguez-Montealegre *et al.*, 2006). This can be attributed not only to the variety but also to the analytical methods used in the studies (Rusjan *et al.*, 2012). Only the most severe CT significantly increased the content of caftaric acid and THCA and the content and proportion of *p*-coumaroyl pentose in berry skin and wine.

Flavanols are a group of phenolic compounds, contributing mostly to wine taste (Rodríguez-Montealegre *et al.*, 2006). Total flavanols contents (TFA) seemed not to be affected by cluster thinning. The most abundant flavanols in grape skin were procyanidin trimer and catechin and in wine procyanidin dimer 2 and epicatechin (Table 4). This is not in agreement with Francesca *et al.* (2014). The content of catechin in 'Blauer Portugieser' berry skin was 8-fold higher than reported for 'Merlot' (Rodríguez-Montealegre *et al.* 2006), 5-fold higher than for 'Syrah' (Peña-Neira *et al.*, 2007), and approximately 10-fold higher than for 'Cabernet Sauvignon' (Lorrain *et al.*, 2011), which can be assigned to varietal characteristics. Only CT2 significantly affected catechin content in berry skin. In wine, CT2 significantly decreased the percentage of catechin and procyanidin trimer among TFA. Furthermore, higher contents of TFA have been measured in grape compared to wine of 'Blauer Portugieser', which can be linked to the degradation of the skin cell wall by pectolytic enzymes and greater permeability of the cell wall during vinification (Ortega-Regules *et al.*, 2006). Moreover, a lower TFA in wine could be also attributed to lower alcohol contents and shorter maceration time, which, according to González-Manzano *et al.* (2004), has a great impact on flavanol extraction from skin to wine. The contents of flavanols in Blauer Portugieser wine are comparable to the data on Blauer Portugieser wine reported by Pour-Nikfardjam *et al.* (2006).

Flavonols are a group of phenolic compounds with a role in antioxidant activity and as UV screening copigments (UV-A and UV-B). In red wines they are usually masked by anthocyanin pigments (Castillo-Muñoz *et al.*, 2007). Fang *et al.* (2013) reported that the presence and amount of individual flavonols depend on berry development and maturity, and that ripe berries contain higher levels of quercetin and myricetin. This was partly confirmed in our study. Among flavonols, quercetin-3-glucuronide, quercetin-3-glucoside and myricetin-3-glucoside were the most abundant in grape skin. In Blauer Portugieser wine, quercetin-3-glucuronide, laricitrin-3-glucoside and myricetin-3-glucoside prevailed. This is comparable to the data of Castillo-Muñoz *et al*. (2007) for 'Cabernet Sauvignon', 'Merlot' and 'Syrah'. According to the proportion of TFO, CT

significantly increased three of the sixteen identified flavonols in grape and four of the thirteen identified flavonols in wine of 'Blauer Portugieser'. On the other hand, we have to underline that flavonol accumulation in berry skin mostly depends on the exposure of clusters to sunlight and that shaded clusters usually contain lower levels of flavonols, as reported by Price *et al.* (1995) and Castillo-Muñoz *et al*. (2007). Additionally, Price *et al.* (1995) reported a 10-fold higher flavonol content in sunlight-exposed clusters of 'Pinot Noir', compared to shaded ones. CT provides higher sunlight exposure of clusters, as leaves in the bunch zone are removed (Reynolds *et al.*, 2004). Consequently, flavonol contents are higher in exposed clusters, which was also observed in our study, especially in CT2. Furthermore, a 1- to 2-fold higher TFO content has been determined in berries of 'Blauer Portugieser' compared to 'Malbec' after CT at the pea-size phenological stage (Fanzone *et al.*, 2011). Similarly, Makris *et al.* (2006) reported lower levels of quercetin and myricetin glycosides (by 16 to 35 mg/kg) in red berry skin compared to our results, especially in grapes from CT vines.

Anthocyanins are the main group of red pigments and copigments in ripening grape of red grapevine varieties (Mazza *et al.*, 1999) directly affecting the colour and sensorial attributes of wine (Gao *et al.*, 1997; Heredia *et al.*, 1998). In general, the contents of the analyzed anthocyanins in grape and wine are in agreement with the anthocyanin content reported in Portugieser wine by Pour-Nikfardjam *et al.* (2006) and in other varieties by Lorrain *et al*. (2011). Glycosylated malvidin, petunidin, peonidin, cyanidin and delphinidin compounds are often present in *V. vinifera* L. grapes and wine (Mazza *et al.*, 1999; García-Beneytez *et al.*, 2002). This was confirmed in 'Blauer Portugieser' berry skin, but in wine delphinidin and cyanidin glycosides were not identified, which can be attributed to varietal characteristics (García-Beneytez *et al.*, 2002). On the other hand, five different malvidin glycosides were identified and quantified in Blauer Portugieser wine that were not present in berry skin. Similarly, Revilla *et al*. (1999) reported several anthocyanin compounds in berry skin but not in wine. Gao *et al.* (1997) reported that the most abundant anthocyanins in grape and wine are malvidin-3-glucoside and their acetylated and coumaroylated glycosides, which was also observed in our study, especially in control grape and wine from CT treatments. Furthermore, King *et al.* (2012) claimed that the proportion of malvidin-3-glucoside regarding TAC significantly increased in wine from CT vines, which is not in accordance with our results. Nevertheless, we have to underline that the proportion of malvidin-3-glucoside acetate, malvidin-3-glucoside pyruvate, malvidin-3-(6″-*p*-coumaroyl) glucoside and malvidin-3-(6″-*p*-coumaroyl) glucoside pyruvate was significantly increased in wine produced from CT treatments. Severe CT significantly increased TAC (8.3 g/kg) in 'Blauer Portugieser' berry skin. It contained higher levels compared to 'Syrah' (Peña-Neira *et al.*, 2007) or 'Merlot' (Bubola *et al.*, 2011) berry skin subjected to CT. Río Segade *et al.* (2008) reported a decline of phenolic concentration in berry skin after phenolic maturity, which has been observed also in our study with CT. A reduction in wine anthocyanin content was observed compared to berry skin, which is in accordance with a report by Rodríguez-Montealegre *et al.* (2006). The amount of extracted phenolics during vinification mostly depends on berry skin cell wall composition and thickness, pectin degradation, alcohol content and maceration period (González-Manzano *et al.*, 2004). Pectin and cellulose, the main components of the cell wall, are degraded by pectinase during the ripening and vinification processes and the result is the release of phenolics into the must (Río Segade *et al.*, 2008). CT could have modified the ripening process of the berries and indirectly influenced the cell wall composition of berry skin. The 'Blauer Portugieser' variety is characterized by high contents of phenolics; therefore, the produced wines are richer in health-promoting compounds (Pour-Nikfardjam *et al.*, 2006). Cluster thinning significantly altered TPC of Blauer Portugieser wine and a similar response has also been reported by Fanzone *et al.* (2011). Heredia *et al.* (1998) reported that wine colour is firstly conditioned by the composition and the content of anthocyanins and secondly by pH, and that anthocyanins in a pH lower than 4.0 yield red to blue tonality (malvidin – red-purple hues, petunidin – purple hues, delphinidin – pink hues, and peonidin – blue-purple hues). Hermosín-Gutiérrez *et al.* (2005) reported that the intense purple-blue tonality of Cabernet Sauvignon wine could be influenced by delphinidin and peonidin contents in grape skin. Anthocyanin polymers are namely less resistant to browning and red colour fading caused by higher pH (Mazza *et al.*, 1999). Acetylated and coumaroylated forms of malvidin and peonidin have been detected in the skin of 'Blauer Portugieser' and in a wider range in wine, which influences the colour of wine (Lorrain *et al.*, 2011). The changes in the proportion of anthocyanins in terms of TAC of 'Blauer Portugieser' suggest that severe CT causes a more purple-blue grape and wine colour compared to the control. However, anthocyanins in berry skin are usually found as monoglycosides, but in must and wine they form into polymeric complexes.

Consequently, the tonality of wine is difficult to predict (Gao *et al.*, 1997).

CONCLUSIONS

Research findings indicated that the impact on yield and grape and wine composition of cluster thinning should not be generalised to all grapevine varieties. Only severe cluster thinning significantly reduced yield (per vine and per hectare) and increased soluble solids content in grape and pH and total extracts in wine. The first report of the detailed profile of phenolics in 'Blauer Portugieser' suggests that anthocyanins prevail in berry skin and wine, followed by flavonols and flavanols in berry, and flavanols and flavonols in wine. Only severe cluster thinning significantly increased the contents of total anthocyanins, flavonols, hydroxycinnamic acids, but not flavanols, in grape and wine. On the other hand, severe cluster thinning modified the proportion of individual phenolics in the corresponding phenolic group, especially of anthocyanins in grape and wine, flavonols in grape, and flavanols in wine. The most abundant hydroxycinnamic acids in 'Blauer Portugieser' grape and wine were caftaric acid and *p*-coumaroyl pentose. Their contents significantly increased following CT2. Severe cluster thinning significantly increased the content and the proportion of catechin in berry skin, and epicatechin and procyanidin dimer 1 in wine. Severe cluster thinning caused several alterations in the flavonol group, especially in wine, where contents and proportions of 3-glucosides of myricetin, laricitrin and quercetin significantly increased. An interesting response of the most abundant flavonol in berry skin and wine (i.e., quercetin-3-glucuronide) was detected after CT2. The content of quercetin-3-glucoside significantly increased, but the proportion regarding TFO decreased. CT also affected the levels of individual anthocyanins, but the response was quite different in berry skin compared to the wine. Severe cluster thinning significantly increased the contents and proportions of 3-glucosides of delphinidin, petunidin and peonidin in berry skin but not in wine. However, cluster thinning significantly increased the amounts and proportions of most identified malvidin-acetylated and coumaroylated glycosides in wine. The results of the study encourage producers of 'Blauer Portugieser' grape and wine to perform severe cluster thinning at pea-size berry phenological stage, so as to enhance grape and wine quality, setting aside the lower yield per vine.

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