

Article

# Effect of Vintage and Viticultural Practices on the Phenolic Content of Hybrid Winegrapes in Very Cool Climate

Mariana Maante-Kuljus <sup>1,\*</sup>, Reelika Rätsep <sup>2,3</sup>, Ulvi Moor <sup>1</sup>, Leila Mainla <sup>1</sup>, Priit Põldma <sup>1</sup>, Angela Koort <sup>1</sup> and Kadri Karp <sup>1</sup>

<sup>1</sup> Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia; ulvi.moor@emu.ee (U.M.); leila.mainla@emu.ee (L.M.); priit.poldma@emu.ee (P.P.); angela.koort@emu.ee (A.K.); kadri.karp@emu.ee (K.K.)

<sup>2</sup> Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre, 69108 Polli, Estonia; reelika.ratsep@emu.ee

<sup>3</sup> Institute of Veterinary Medicine and Animal Sciences, ERA-Chair Valortech, Kreutzwaldi 56/5, 51014 Tartu, Estonia

\* Correspondence: mariana.maante-kuljus@emu.ee

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**Abstract:** Vine growing and wine production is gaining in popularity around the Baltic Sea Region. The first aim of the experiment was to determine the variability of the total phenolic and anthocyanin content (from 2010 to 2018) and of individual anthocyanin content (from 2016 to 2018) in the hybrid grape cultivars ‘Hasansky Sladky’, ‘Zilga’, and ‘Rondo’. In field conditions ‘Rondo’ had winter cold damage to canes in two years. Therefore, the second aim was to determine the effect of high polyethylene tunnel and field conditions on fruit total and individual anthocyanin content of ‘Rondo’ from 2016 to 2018. Over nine years, the total phenolic content ranged from 192 to 671 mg 100 g<sup>-1</sup> and anthocyanins from 30 to 405 mg 100 g<sup>-1</sup> spectrophotometrically. The anthocyanin (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside) content depended on cultivar properties and climatic parameters. Antioxidant activity was cultivar dependent and ranged from 40 to 88%. Poly tunnel cultivation increased the content of total anthocyanins in ‘Rondo’ from 447 to 1472 mg 100 g<sup>-1</sup> (by chromatographically) in cooler year, but in warmer years it decreased from 3645 to 1618 mg 100 g<sup>-1</sup>. Individual anthocyanins showed the same tendency. Grapevine cultivar ‘Rondo’ is recommended for tunnel growing in very cool climate conditions.

**Keywords:** anthocyanins; antioxidant activity; cyanidin; delphinidin; malvidin; peonidin; petunidin; *Vitis* hybrids

## 1. Introduction

Polyphenols are important for wine color intensity, astringency, and bitterness. The phenolic content and profile of grapes depends on cultivar, growing area, climatic conditions, and viticultural practices [1–7]. Cultivars with a higher total phenolic content tend to have higher antioxidant activity [8,9]. Anthocyanins are the main compounds responsible for the red color of grapes and they are synthesized to protect the skin from the negative effect of the environment, especially ultraviolet radiation. The anthocyanin composition depends on the genetic background of *Vitis* species [10]. However, it has also been found to be affected by cultivar, climate [11], and different viticultural practices [12,13]. Elevated temperatures during ripening may reduce the accumulation of anthocyanins and could partly degrade the previously synthesized components [14,15]. Therefore,

anthocyanin accumulation in hot regions is inhibited in the skins of red and black grapes, but further north conditions are more favorable. In wines produced from hybrid cultivars, malvidin was the most abundant compound, accounting for 58 to 62% of the pigments [16]. The anthocyanin profile of wine has been used as a tool to assess the varietal origin of single cultivar wines, being called the “anthocyanin fingerprint” [17]. Hybrids differ in their phenolic and anthocyanin profiles from *Vitis vinifera* cultivars [2–4]. So far the phenolic compounds of cultivars ‘Hasansky Sladky’ and ‘Zilga’ have been found to be affected by viticultural practices such as defoliation [18] and pruning methods [19]. There are no long-term studies on how very cool climatic conditions affect the phenolic composition of cultivars ‘Hasansky Sladky’, ‘Zilga’, and ‘Rondo’.

Hybrid grape cultivars are suitable for growing in cool climate conditions [20]. In Finland, ‘Zilga’ and ‘Rondo’ have been tested in the field [21,22] and have potential as high quality wine grapes. In Estonia, vine growing has intensified in recent years and winemaking even more. According to the Heliothermal Index, Estonia belongs to a very cool vine growing area [23]. In this region, ‘Hasansky Sladky’, and ‘Zilga’ in the field reached desired soluble solids content, but ‘Rondo’ did not.

The first aim of this study was to determine the variability of the total phenolic and anthocyanin content over a period of 2010–2018 and of individual anthocyanin content over the years 2016–2018 in the hybrid grape cultivars ‘Hasansky Sladky’, ‘Zilga’, and ‘Rondo’. The second aim was to determine the effect of high polyethylene tunnel and field conditions on fruit total and individual anthocyanins over the years 2016–2018 in ‘Rondo’.

## 2. Materials and Methods

### 2.1. Experimental Sites and Plant Material

‘Hasansky Sladky’, ‘Zilga’, and ‘Rondo’ were investigated in field conditions in a period of 2010–2018. The effect of polytunnel viticultural practice in ‘Rondo’ was investigated in 2016–2018 period. The polytunnel (58°17′1″ N, 26°33′41″ E) was 28 m long, 7.6 m wide, and 4.6 m high, covered with 0.18 mm thick UV stable low-density polyethylene (direct photosynthetically active radiation 88–90) and was situated 8.5 km from the field vineyard (58°17′1″ N, 26°33′41″ E). At both sites, the vine rows were oriented from north to south. Vines of tunnel were planted in spaces of 1.6 × 2 m and in field 2 × 2 m. Both vineyards were established in 2007 with own-rooted plants. The experimental design was randomized block with 4 replicates and 8 vines in each. In 2010, field grown ‘Zilga’ had spring frost damage to flowers, and ‘Rondo’ had winter cold damage to canes in 2013 and 2014. In table “x” marks years without harvest in these cultivars.

The soil of both experimental areas was sandy loam *Haplic Luvisol*. Soils were sufficiently drained and soil fertility was 45 to 50 points in 100-point scale. The soil nutrient content in the field was: P and Mg—excessive, K—high, Ca—medium and pH<sub>KCl</sub> was 5.4 (Table 1). P, K, Ca, and Mg values in the tunnel were high and pH<sub>KCl</sub> was 5.4.

**Table 1.** Nutrient content (mg kg<sup>-1</sup>) of the soil in two experimental areas.

Viticultural Practice	P	K	Mg	Ca	pH <sub>KCl</sub>
Field	147	257	260	1670	5.4
Tunnel	159	578	574	2381	5.4

Experimental cultivars:

‘Hasansky Sladky’ (*Vitis amurensis* Ruprecht × ‘Dalnevostochnyi Tikhonova’) [24] (synonyms: ‘Hasan Sweet’, ‘Varajane Sinine’, ‘Baltica’) is a vigorous Russian winegrape cultivar which ripens exceptionally early. It has long, small to medium-sized slightly loose clusters and small-medium blue-black berries. It is quite disease resistant and has good winter hardiness.

'Zilga' [(‘Smuglyanka’ × ‘Dvietes’) × ‘Jublinaja Novgoroda’] [25] is a Latvian early ripening wine and table grape cultivar. It has small to medium semi-tight clusters and medium blue to sky-blue shade berries. It is a very vigorous and productive vine.

'Rondo' ('Zarya Severa' × 'Saint Laurent') [26] is a German wine and table grape cultivar with medium ripening yield. It has medium sized blue berries and clusters. Plant growth is vigorous.

## 2.2. Vineyard Management

For establishment of the experiments at both sites the ground was covered with black synthetic mulch, and no irrigation system was established. No additional fertilizers were used at either experimental area. White polypropylene fabric was used in tunnel as winter cover. Vines were trained on low double trunk trellis with 12 buds left per plant. Branches were pruned after sap flow was ended in spring. After formation of inflorescence, shoots were thinned (fruitless shoots were removed). Once every two weeks, lateral shoots and main shoots were cut back. Shoots height was eight leaves after clusters. About four leaves from the cluster zone were removed at the beginning of *veraison*. After *veraison* soluble solids content was determined once a week on randomly chosen grapes from basal clusters. The harvest time was determined when soluble solids content did not change significantly or due to the weather conditions (arrival of night frosts). Depending on year, the technological maturity parameters at harvest in field conditions were: 'Hasansky Sladky'—soluble solids content (SSC) 17 to 21 °Brix, titratable acids content (TAC) 6.5 to 20.7 G tartaric acid L<sup>-1</sup>, pH 2.9 to 3.6; 'Rondo' SSC 12 to 17 °Brix, TAC 9.2 to 22.7 G tartaric acid L<sup>-1</sup>, pH 2.8 to 3.6; 'Zilga' SSC 13 to 19 °Brix, TAC 6.7 to 17.6 G tartaric acid L<sup>-1</sup>, pH 2.8 to 3.5 [23]. In tunnel conditions: 'Rondo' SSC was 15 to 18 °Brix, TAC 6.5 to 8.3 G tartaric acid L<sup>-1</sup>, pH 3 to 3.4. The average weight of 'Hasansky Sladky' berry was 1.3 g and cluster 52 g, 'Rondo' respectively 2.1 g and 119 g, and 'Zilga' respectively 2.4 g and 138 g.

## 2.3. Weather Conditions

Weather data was obtained from The Estonian Environment Agency Tartu-Tõravere meteorological station (located 11 km from the field and 6 km from tunnel). The air temperatures, precipitation, and relative air humidity (RH, %) were recorded in the observation area 24 h a day, every hour. The tunnel air temperature was recorded with temperature data loggers 24 h a day, every hour. In most experimental years, the warmest month was July, except in 2015 and 2017 when August was warmer (Table 2). In spring months the mean temperature in May was 12.5 °C and in June 15.2 °C. At the time of *veraison* mean temperature in July was 18.5 °C, in August 16.7 °C and in September 12.2 °C. Mean monthly temperatures in 2018 were higher in every month compared to the mean of experimental years. Temperatures in tunnel conditions were higher—depending on month 0.6 to 5.9 °C. There was a drought in May 2016 and 2018. Autumn months in 2016 and 2017 differed from other years by the severity of precipitation. Additionally, the air humidity was higher in these years.

The sum of active temperatures (SAT) was calculated by summing the daily average temperatures above 10 °C (monthly, year). The grape phenological growth stage identification scale (BBCH) was used in phenological observations [27]. Phenological observations were made once a week over the vegetation period (April to October) on the basal cluster of each plant. Over the period of grape development (SAT BBCH 71–79, June–July) and at the time of *veraison* (SAT BBCH 81–89, August–September) SAT was calculated. The radiation flux (RF, W m<sup>2</sup>) data was obtained from the Tartu University Laboratory of Environmental Physics (10 km from field and 12 km from tunnel). RF was recorded in the observation area 24 h a day, every five minutes. The monthly average was calculated. RF for the years from 2016 to 2018 was 153 to 183 W m<sup>2</sup> in August and in September 93 to 120 W m<sup>2</sup>.

**Table 2.** Monthly temperatures, precipitation and relative air humidity from April to October in the years from 2010 to 2018 in field conditions and the monthly temperatures in tunnel from 2016 to 2018.

		Mean Temperature (°C)						
	Year	April	May	June	July	August	September	October
Field	2010	5.7	12.2	14.3	<b>21.7</b>	<b>17.8</b>	10.7	3.8
	2011	5.7	11.0	<b>17.2</b>	<b>19.9</b>	15.8	<b>12.3</b>	<b>6.8</b>
	2012	4.6	11.4	13.3	17.7	14.7	11.9	5.3
	2013	4.0	<b>15.5</b>	<b>17.8</b>	17.5	16.6	10.8	<b>6.6</b>
	2014	<b>6.8</b>	12.3	13.7	<b>19.5</b>	<b>16.8</b>	<b>12.5</b>	<b>5.6</b>
	2015	<b>5.8</b>	10.6	14.6	16.1	<b>17.0</b>	<b>12.8</b>	4.9
	2016	<b>5.9</b>	<b>14.1</b>	<b>16.3</b>	18.2	16.3	<b>12.5</b>	4.2
	2017	1.6	10.2	13.8	15.7	16.5	12.1	5.2
	2018	<b>7.2</b>	<b>15.2</b>	<b>15.5</b>	<b>20.2</b>	<b>18.5</b>	<b>14.0</b>	<b>7.2</b>
	Mean	5.3	12.5	15.2	18.5	16.7	12.2	5.5
Tunnel	2016	6.9	18.0	<b>20.5</b>	22.0	18.6	13.4	5.2
	2017	4.6	16.1	17.1	20.6	18.8	12.7	6.0
	2018	<b>10.2</b>	<b>20.1</b>	<b>20.4</b>	<b>23.6</b>	<b>20.8</b>	<b>15.6</b>	<b>8.6</b>
	Mean	7.2	18.1	19.3	22.1	19.4	13.9	6.6
		Precipitation (mm)						
Field	2010	25	<b>97</b>	<b>98</b>	38	<b>148</b>	<b>99</b>	<b>59</b>
	2011	1	<b>58</b>	35	48	55	<b>80</b>	48
	2012	<b>45</b>	<b>78</b>	<b>98</b>	<b>80</b>	80	61	<b>72</b>
	2013	36	<b>65</b>	29	<b>67</b>	73	38	45
	2014	16	<b>90</b>	<b>134</b>	<b>78</b>	<b>126</b>	20	43
	2015	<b>80</b>	<b>61</b>	66	<b>68</b>	47	<b>67</b>	8
	2016	<b>70</b>	2	<b>207</b>	<b>86</b>	<b>104</b>	15	37
	2017	29	28	65	57	<b>112</b>	<b>119</b>	<b>86</b>
	2018	<b>43</b>	10	66	23	81	<b>99</b>	<b>78</b>
	Mean	38	54	89	61	92	64	53
		Relative air humidity (%)						
Field	2010	68	<b>72</b>	<b>72</b>	68	<b>79</b>	<b>84</b>	83
	2011	65	<b>66</b>	65	72	75	81	81
	2012	65	61	69	73	<b>81</b>	<b>84</b>	<b>91</b>
	2013	<b>69</b>	<b>69</b>	<b>71</b>	72	73	80	84
	2014	53	<b>69</b>	<b>76</b>	70	76	77	78
	2015	<b>75</b>	<b>71</b>	70	77	75	<b>86</b>	84
	2016	<b>73</b>	61	<b>71</b>	<b>80</b>	<b>83</b>	<b>84</b>	84
	2017	<b>75</b>	62	<b>71</b>	<b>76</b>	77	<b>85</b>	<b>87</b>
	2018	67	54	63	73	74	81	<b>88</b>
	Mean	68	65	70	73	77	82	84

Note: Data according to the Estonian Environment Agency from Tartu-Tõravere weather station. Tunnel temperature data were collected from temperature data logger. Results in bold are higher than the mean of all experimental years.

The Heliothermal Index (HI) was calculated using the following expression (Huglin [28]):

$$HI = \sum_{Mi}^{Mf} \left[ \frac{(T - 10) + (T_{max} - 10)}{2} \right] \times d \quad (1)$$

where ' $T$ ' and ' $T_{max}$ ' are, the average mean and maximum monthly temperature (°C), respectively; ' $Mi$ ' and ' $Mf$ ' are the initial and the final month of the period, respectively; ' $d$ ' is the length of day coefficient, with value of 1.09 for latitudes 58°.

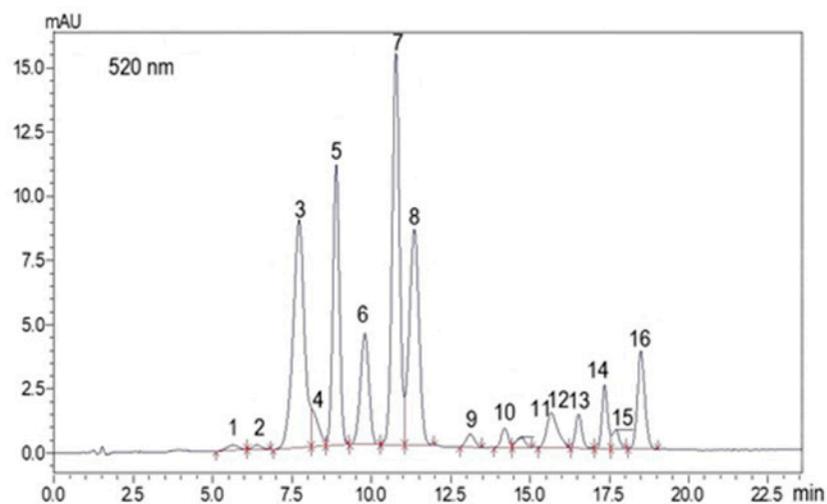
#### 2.4. Measurements and Analysis

Samples of grapes were collected from all experimental cultivars at harvest from the field in 2010 to 2018 and from 'Rondo' in the tunnel in 2016 to 2018. For phenolic compounds samples (three replications) of 400 g from the different parts of the basal cluster for analyses were collected from

every cultivar/viticultural practice. From one replication three separate extractions were made from grape skins (exocarp of fruit). From 2010 to 2018, at harvest, samples were collected from the field.

The total phenolic content (TPC) was determined by applying the Folin-Ciocalteu phenol reagent method [29]. Ethanol-acetone (7:3) solution was used as the solvent to extract the total phenolic compounds (5 g of berries skins was added 50 mL of solution). TPC was expressed as mg of gallic acid equivalent per 100 g of fresh skins weight (FSW). Total anthocyanin content ( $ACC_{spec}$ ) was determined with the pH-differential method [29]. Hydrochloric acid-ethanol (15:85) solution was used as the solvent to extract the  $ACC_{spec}$  (10 g of berry skins was added 100 mL of solution).  $ACC_{spec}$  was expressed as mg of cyanidin-3-glucoside equivalent per 100 g of FSW. In 2016 to 2018 samples, the total antioxidant activity (TAA) was determined by applying the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [30]. TAA was expressed as %. Spectrophotometric measures were made with UVmini-1240 Shimadzu (Shimadzu, Kyoto, Japan).

Total anthocyanin ( $ACC_{HPLC}$ ) and individual anthocyanins (Figure 1) were determined using the method for polyphenol profiling [31]. Samples were prepared in three replicates; approx. 1 g of berry skin sample was added to 50% ethanol + 1% HCl (v:v) solution. Chromatographic analyses (HPLC) were made with Shimadzu Nexera X2 (Shimadzu, Kyoto, Japan) at a wavelength of 520 nm. The results of total  $ACC_{HPLC}$  were expressed as malvidin-3-O-glycoside equivalent mg 100 g<sup>-1</sup> of FSW. The major anthocyanins were delphinidin-3-O-glucoside (Dp), cyanidin-3-O-glucoside (Cy), petunidin-3-O-glucoside (Pt), peonidin-3-O-glucoside (Pn), and malvidin-3-O-glucoside (Mv).



**Figure 1.** An example of anthocyanins profile of ‘Rondo’: 1—cyanidin-3-O-glucoside-5-O-glucoside; 2—petunidin-3-O-glucoside-5-O-glucoside; 3—delphinidin-3-O-glucoside; 4—malvidin-3-O-glucoside-5-O-glucoside; 5—cyanidin-3-O-glucoside; 6—petunidin-3-O-glucoside; 7—peonidin-3-O-glucoside; 8—malvidin-3-O-glucoside; 9—delphinidin-3-O-(6''-O-acetyl)-glucoside; 10—cyanidin-3-O-(6-O-acetyl)-glucoside; 11—petunidin-3-O-(6''-O-acetyl)-glucoside; 12—malvidin-3-O-(6-O-acetyl)-glucoside; 13—delphinidin-3-O-(6''-p-coumaroyl)-glucoside; 14—cyanidin-3-O-(6''-p-coumaroyl)-glucoside; 15—petunidin-3-O-(6''-O-coumaroyl)-glucoside; 16—peonidin-3-O-(6''-O-coumaroyl)-glucoside.

## 2.5. Statistical Analysis

The results of measured parameters for ‘Hasansky Sladky’, ‘Rondo’ and ‘Zilga’ were tested by one-way analysis of variance. The least significant difference ( $LSD_{0.05}$ ) was calculated to evaluate the effect of year and different letters in tables mark significant differences at  $p \leq 0.05$ . The effect of cultivar was tested by one-way analysis of variance. To evaluate the main effect of two factors (experimental, year and cultivar and the interaction between them), the two-way analyses of variance was carried out, and results marked as non-significant (ns) or using confidence level significance at  $p \leq 0.05$  \*,  $p \leq 0.01$  \*\* and  $p \leq 0.001$  \*\*\*. The effect of treatment in the ‘Rondo’ viticultural practice experiment was

tested by one-way analysis of variance. To evaluate the main effect of two factors (year and viticultural practice and the interaction between them), the two-way analyses of variance was carried out, and results marked as non-significant (ns), or using confidence level significant at  $p \leq 0.05$  \*,  $p \leq 0.01$  \*\* and  $p \leq 0.001$  \*\*\*. Linear correlation coefficients were calculated between the variables ( $n = 9$  'Hasansky Sladky', 'Rondo', 'Zilga') with coefficient significance being  $p \leq 0.05$  \*, and  $0.01$  \*\*. Relationship strength was estimated  $0.3 \leq r \leq 0.7$  moderate, and  $r \geq 0.7$  strong. Principal component analysis (PCA) was applied to the chemical composition of grapes and meteorological data to study the possible grouping of the cultivars and year.

### 3. Results

#### 3.1. Climate Conditions

The average SAT for the BBCH 71–79 was 1031 °C and ranged from 877 to 1167 °C (Table 3). For the SAT BBCH 81–89 the average was 851 °C, ranging from 789 to 938 °C. The average SAT for the years from 2010 to 2018 was 2320 °C and ranged from 1981 to 2660 °C. Four years out of nine the SAT exceeded the mean value of all years. HI had large variability in test years and ranged from 888 to 1532. Estonia belongs to in a very cool vine growing region, except in 2018, when HI was above 1500 and according to this Estonia could be classified to cool region. The average length of the frost-free period was 158 days, ranging from 140 to 180 days. The last spring frost usually occurred at the beginning of May, except in 2017 when it was in mid-May. The first autumn frost occurred mostly in the second half of October, but in four years, it was at the end of September or beginning of October. The amount of precipitation ranged from 325 to 566 mm and the rainiest years was 2010.

**Table 3.** The sum of active temperatures, Heliothermal Index, frost-free period, and the precipitation (April–October, 2010–2018).

Year	SAT (°C)			HI	Frost-Free Period (Days)	Precipitation (mm)
	BBCH 71–79	BBCH 81–89	Total			
2010	<b>1148</b>	834	<b>2331</b>	<b>1375</b>	148	<b>566</b>
2011	<b>1167</b>	<b>889</b>	<b>2498</b>	<b>1366</b>	<b>162</b>	325
2012	957	789	2181	1037	<b>172</b>	<b>514</b>
2013	<b>1103</b>	826	<b>2490</b>	<b>1308</b>	150	352
2014	979	<b>854</b>	2274	<b>1234</b>	140	<b>507</b>
2015	937	<b>877</b>	2156	1078	149	398
2016	1025	824	2311	<b>1279</b>	<b>165</b>	<b>520</b>
2017	877	831	1981	888	156	<b>497</b>
2018	<b>1086</b>	<b>938</b>	<b>2660</b>	<b>1532</b>	<b>180</b>	399
Mean	1031	851	2320	1233	158	453

Note: SAT—sum of active temperatures ( $\geq 10$  °C); BBCH – phenological growth stage identification scale; BBCH 71–79—active temperatures of June, July; BBCH 81–89—active temperatures of August, September; HI—Heliothermal Index. Data according to the Estonian Environment Agency from Tartu-Tõravere weather station. Results in bold are higher than the mean of experimental years.

#### 3.2. Phenolic Compounds

The TPC had a large variation due to the effect of year—TPC in 'Hasansky Sladky' ranged from 192 to 394 mg 100 g<sup>-1</sup>, in 'Rondo' from 374 to 671 mg 100 g<sup>-1</sup> and in 'Zilga' from 214 to 372 mg 100 g<sup>-1</sup> (Table 4). Each cultivar had its highest content in a different year—'Hasansky Sladky' in 2011, 'Rondo' in 2018, and 'Zilga' in 2014 and 2016. The TPC was significantly affected by year, cultivar, and interaction between them ( $p \leq 0.001$ ).

**Table 4.** The effect of year and cultivar on phenolic compounds of grape cultivars ‘Hasansky Sladky’, ‘Rondo’ and ‘Zilga’ (2010–2018) cultivated in field conditions.

Year	‘Hasansky Sladky’		‘Rondo’		‘Zilga’	
	TPC	ACC <sub>spec</sub>	TPC	ACC <sub>spec</sub>	TPC	ACC <sub>spec</sub>
	mg 100 g <sup>-1</sup> FSW					
2010	279 d	133 a	399 cd	112 d	×	×
2011	394 a	74 c	482 b	134 bc	293 b	54 e
2012	192 h	113 d	374 d	75 e	214 e	50 e
2013	326 b	138 a	×	×	222 de	64 d
2014	253 e	30 f	×	×	344 a	32 f
2015	227 f	51 e	391 d	159 bc	273 bc	85 c
2016	211 g	75 d	477 b	183 b	372 a	139 b
2017	293 c	81 d	447 bc	166 b	267 bc	86 c
2018	289 c	118 bc	671 a	405 a	256 cd	150 a
	TPC	ACC <sub>spec</sub>				
Cultivar	***	***				
Year	***	***				
Interaction	***	***				

Note: ×—no results. TPC—total phenol content; ACC<sub>spec</sub>—total anthocyanin content determined spectrophotometrically; FSW—fresh skins weight. Different letters in the same columns among years mark significant differences at  $p \leq 0.05$ . The main effect of the year and cultivar and their interaction, \*\*\*—significant at  $p \leq 0.001$ .

Among nine years, the ACC<sub>spec</sub> was significantly different—the contents ranged from 30 to 405 mg 100 g<sup>-1</sup> (Table 4). In ‘Hasansky Sladky’, the ACC<sub>spec</sub> ranged from 30 to 138 mg 100 g<sup>-1</sup> and the highest content was determined in two experimental years out of nine (2010 and 2013). In ‘Rondo’, ACC<sub>spec</sub> ranged from 75 to 405 mg 100 g<sup>-1</sup> and significantly highest content was found in 2018. In ‘Zilga’, the content ranged from 32 to 150 mg 100 g<sup>-1</sup> and the highest ACC<sub>spec</sub> was in one year out of eight (2018). The ACC<sub>spec</sub> was significantly affected by year, cultivar, and interaction between them ( $p \leq 0.001$ ).

### 3.3. Antioxidant Activity and Anthocyanins

In all experimental years, TAA in ‘Rondo’ was significantly higher compared to the other cultivars (ranged between 78 and 88%) (Table 5). TAA in ‘Hasansky Sladky’ and ‘Zilga’ did not differ significantly in two out of three years. TAA in ‘Hasansky Sladky’ ranged from 40 to 53% and in ‘Zilga’ from 53 to 62%. The TAA was significantly affected by cultivar ( $p \leq 0.001$ ) and interaction between cultivar and year ( $p \leq 0.01$ ).

In 2016 and 2018, all individual anthocyanins were significantly higher in ‘Rondo’ (Table 5). Dp and Cy contents were significantly lower in ‘Hasansky Sladky’ in all experimental years. Pn did not differ significantly between cultivars ‘Hasansky Sladky’ and ‘Zilga’. Mv and Pt content differed significantly between cultivars and years. Individual anthocyanins were significantly affected by year, cultivar and the interaction between them ( $p \leq 0.001$ ).

Viticultural practice caused significant variability in the content of ACC<sub>HPLC</sub> and individual anthocyanins (Table 6). TAA was significantly affected by the viticultural practice ( $p \leq 0.01$ ) and interaction of year and viticultural practice ( $p \leq 0.05$ ). ACC<sub>HPLC</sub> ranged from 447 to 3645 mg 100 g<sup>-1</sup> in the field and from 1108 to 1618 mg 100 g<sup>-1</sup> in the tunnel. ACC<sub>HPLC</sub> and individual anthocyanins were significantly higher in the grapes grown in the field in 2016 and 2018. In 2017, the tunnel grown grapes had higher individual anthocyanin content, Dp by 78%, Cy by 26%, Pt by 81%, Pn by 21%, and Mv by 77%. ACC<sub>HPLC</sub> and individual anthocyanins were significantly affected by year, cultivar and the interaction between them, except for Dp the viticultural practice did not have a significant effect.

**Table 5.** The effect of cultivar and year on antioxidant activity and the content of individual anthocyanins' in grapes of 'Hasansky Sladky', 'Rondo', and 'Zilga' (2016–2018) cultivated in field conditions.

Year	Cultivar	TAA %	mg 100 g <sup>-1</sup> FSW				
			Dp	Cy	Pt	Pn	Mv
2016	'Hasansky Sladky'	40 c	18 c	10 c	20 c	14 b	59 c
	'Rondo'	88 a	311 a	190 a	157 a	302 a	298 a
	'Zilga'	62 b	145 b	90 b	57 b	19 b	75 b
2017	'Hasansky Sladky'	53 b	39 c	23 c	25 c	21 b	41 c
	'Rondo'	78 a	87 b	64 a	33 b	102 a	71 a
	'Zilga'	53 b	111 a	28 b	44 a	21 b	52 b
2018	'Hasansky Sladky'	51 b	111 c	7 c	90 b	21 b	309 b
	'Rondo'	84 a	1189 a	251 a	416 a	333 a	712 a
	'Zilga'	53 b	449 b	107 b	70 b	5 b	72 c
Year		ns	***	***	***	***	***
Cultivar		***	***	***	***	***	***
Interaction		**	***	***	***	***	***

Note: TAA—total antioxidant activity; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside; FSW—fresh skins weight. Different letters in columns mark significant differences of means among years according to the cultivars at  $p \leq 0.05$ . The main effect of the year, cultivar, and the interaction between them, ns—no significance, \*—significant at  $p \leq 0.01$ , \*\*—significant at  $p \leq 0.001$ .

**Table 6.** The effect of viticultural practice on the total antioxidant activity, total anthocyanins, and individual anthocyanins of 'Rondo' grapes (2016–2018).

Year	Viticultural Practice	TAA %	mg 100 g <sup>-1</sup> FSW					
			ACC <sub>HPLC</sub>	Dp	Cy	Pt	Pn	Mv
2016	Field	88 a	1581 a	311 a	190 a	157 a	302 a	298 a
	Tunnel	89 a	1108 b	230 b	30 b	113 b	51 b	235 b
2017	Field	78 a	447 b	87 b	64 b	33 b	102 b	71 b
	Tunnel	90 a	1472 a	396 a	86 a	171 a	129 a	306 a
2018	Field	84 a	3645 a	1189 a	251 a	416 a	333 a	712 a
	Tunnel	86 a	1618 b	820 b	12 b	107 b	23 b	177 b
Year		ns	***	***	**	***	***	***
Viticultural practice		**	***	ns	***	***	***	***
Interaction		*	***	***	***	***	***	***

Note: TAA—total antioxidant activity; ACC<sub>HPLC</sub>—total anthocyanin content determined by chromatographically; FSW—fresh skins weight; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside. Different letters in the same columns among years according to the viticultural practice mark significant differences of means at  $p \leq 0.05$ . The main effects of the year and viticultural practice and their interaction, ns—no significant, \*—significant at  $p \leq 0.05$ , \*\*—significant at  $p \leq 0.01$ , \*\*\*—significant at  $p \leq 0.001$ .

### 3.4. Correlations

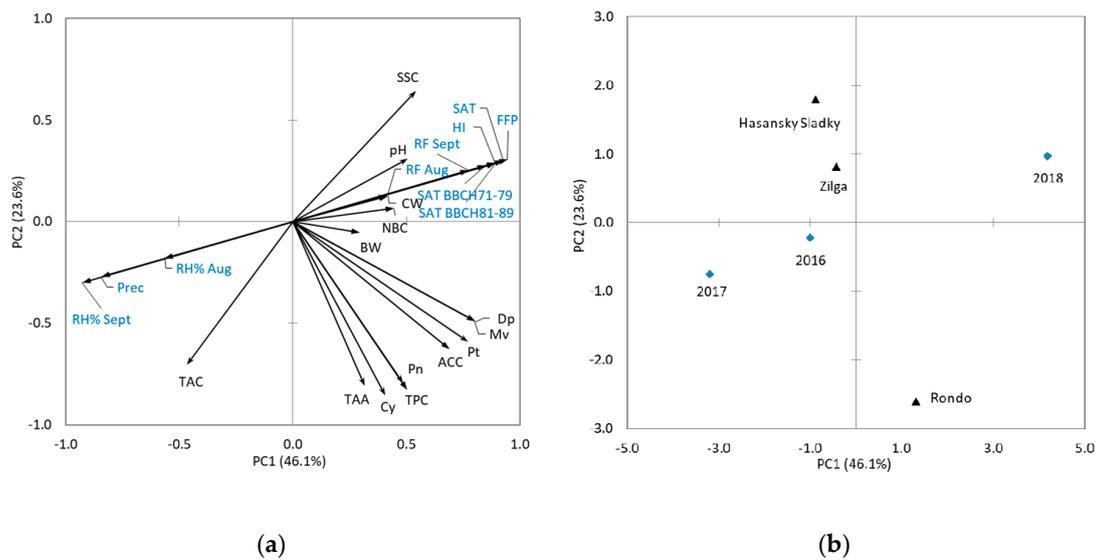
There were significant correlations between climatic data and phenolic compounds (Table 7). In 'Hasansky Sladky', TAA had positive correlation with August RF and negative with August RH. In 'Rondo', TPC had positive correlation with temperature parameters and September RF and negative with precipitation and RH. In 'Hasansky Sladky', TPC correlated only with August RF (positive) and RH (negative). Inversely in 'Zilga', TPC correlated negatively with August RF and positively with precipitation and August RH. In all experimental cultivars, ACC<sub>HPLC</sub>, Dp, Pt, and Mv had positive correlation with most of the temperature parameters and negative with precipitation and RH. In contrast, Cy in 'Hasansky Sladky' and Cy, Pn in 'Zilga' had negative correlation with temperature parameters. In 'Zilga', Cy and Pt had positive correlation with precipitation and RH.

**Table 7.** Correlations between phenolic compounds and climatic data for grape cultivars ‘Hasansky Sladky’, ‘Rondo’, and ‘Zilga’ (2016–2018) in field conditions.

	TAA	TPC	ACC <sub>HPLC</sub>	Dp	Cy	Pt	Pn	Mv
‘Hasansky Sladky’								
SAT BBCH 71–79	−0.306 <sup>ns</sup>	−0.276 <sup>ns</sup>	0.717 *	0.551 <sup>ns</sup>	−0.988 **	0.676 *	−0.233 <sup>ns</sup>	0.759 *
SAT BBCH 81–89	0.400 <sup>ns</sup>	0.505 <sup>ns</sup>	0.992 **	0.973 **	−0.586 <sup>ns</sup>	0.987 **	0.500 <sup>ns</sup>	0.988 **
Year, SAT	−0.087 <sup>ns</sup>	−0.029 <sup>ns</sup>	0.865 **	0.738 *	−0.925 **	0.834 **	0.001 <sup>ns</sup>	0.895 **
HI	−0.211 <sup>ns</sup>	−0.167 <sup>ns</sup>	0.789 *	0.639 <sup>ns</sup>	−0.968 **	0.752 *	−0.130 <sup>ns</sup>	0.826 **
August, RF	0.842 **	0.958 **	0.679 *	0.812 **	0.138 <sup>ns</sup>	0.712 *	0.910 **	0.633 <sup>ns</sup>
September, RF	−0.428 <sup>ns</sup>	−0.415 <sup>ns</sup>	0.606 <sup>ns</sup>	0.423 <sup>ns</sup>	−0.996 **	0.560 <sup>ns</sup>	−0.366 <sup>ns</sup>	0.654 <sup>ns</sup>
Frost-free period	0.026 <sup>ns</sup>	0.097 <sup>ns</sup>	0.920 **	0.815 **	−0.871 **	0.894 **	0.120 <sup>ns</sup>	0.943 **
Precipitation	−0.497 <sup>ns</sup>	−0.608 <sup>ns</sup>	−0.978 **	−0.986 **	0.481 <sup>ns</sup>	−0.979 **	−0.595 <sup>ns</sup>	−0.966 **
August, RH%	−0.774 *	−0.893 **	−0.800 **	−0.901 **	0.045 <sup>ns</sup>	−0.825 **	−0.854 **	−0.763 *
September, RH%	−0.287 <sup>ns</sup>	−0.383 <sup>ns</sup>	−0.989 **	−0.942 **	0.691 *	−0.977 **	−0.387 <sup>ns</sup>	−0.994 **
‘Rondo’								
SAT BBCH 71–79	0.583 <sup>ns</sup>	0.801 **	0.915 **	0.839 **	0.938 **	0.900 **	0.965 **	0.919 **
SAT BBCH 81–89	0.103 <sup>ns</sup>	0.979 **	0.911 **	0.964 **	0.671 *	0.924 **	0.545 <sup>ns</sup>	0.916 **
Year, SAT	0.463 <sup>ns</sup>	0.923 **	0.983 **	0.945 **	0.918 **	0.977 **	0.894 **	0.988 **
HI	0.534 <sup>ns</sup>	0.862 **	0.953 **	0.893 **	0.937 **	0.942 **	0.941 **	0.957 **
August, RF	−0.416 <sup>ns</sup>	0.581 <sup>ns</sup>	0.379 <sup>ns</sup>	0.524 <sup>ns</sup>	0.027 <sup>ns</sup>	0.411 <sup>ns</sup>	−0.171 <sup>ns</sup>	0.382 <sup>ns</sup>
September, RF	0.638 <sup>ns</sup>	0.704 *	0.846 **	0.750 *	0.922 **	0.827 **	0.978 **	0.850 **
Frost-free period	0.390 <sup>ns</sup>	0.963 **	0.994 **	0.977 **	0.886 **	0.992 **	0.837 **	0.999 **
Precipitation	−0.014 <sup>ns</sup>	−0.949 **	−0.854 **	−0.926 **	−0.584 <sup>ns</sup>	−0.871 **	−0.440 <sup>ns</sup>	−0.859 **
August, RH%	0.303 <sup>ns</sup>	−0.719 *	−0.541 <sup>ns</sup>	−0.669 *	−0.198 <sup>ns</sup>	−0.569 <sup>ns</sup>	−0.007 <sup>ns</sup>	−0.545 <sup>ns</sup>
September, RH%	−0.198 <sup>ns</sup>	−0.994 **	−0.957 **	−0.988 **	−0.755 *	−0.965 **	−0.651 <sup>ns</sup>	−0.962 **
‘Zilga’								
SAT BBCH 71–79	0.142 <sup>ns</sup>	0.149 <sup>ns</sup>	0.825 **	0.783 *	−0.838 **	0.954 **	−0.817 **	0.899 **
SAT BBCH 81–89	−0.387 <sup>ns</sup>	−0.608 <sup>ns</sup>	0.967 **	0.987 **	−0.948 **	0.805 **	−0.965 **	0.340 <sup>ns</sup>
Year, SAT	−0.028 <sup>ns</sup>	−0.099 <sup>ns</sup>	0.936 **	0.912 **	−0.939 **	0.976 **	−0.929 **	0.775 *
HI	0.067 <sup>ns</sup>	0.039 <sup>ns</sup>	0.881 **	0.847 **	−0.890 **	0.971 **	−0.874 **	0.850 **
August, RF	−0.663 <sup>ns</sup>	−0.978 **	0.543 <sup>ns</sup>	0.610 <sup>ns</sup>	−0.504 <sup>ns</sup>	0.196 <sup>ns</sup>	−0.547 <sup>ns</sup>	−0.390 <sup>ns</sup>
September, RF	0.240 <sup>ns</sup>	0.294 <sup>ns</sup>	0.733 *	0.682 *	−0.752 *	0.913 **	−0.724 *	0.945 **
Frost-free period	−0.114 <sup>ns</sup>	−0.223 <sup>ns</sup>	0.970 **	0.956 **	−0.968 **	0.963 **	−0.964 **	0.694 *
Precipitation	0.454 <sup>ns</sup>	0.700 *	−0.932 **	−0.961 **	0.909 **	−0.730 *	0.931 **	−0.223 <sup>ns</sup>
August, RH%	0.632 <sup>ns</sup>	0.937 **	−0.685 *	−0.744 *	0.649 <sup>ns</sup>	−0.368 <sup>ns</sup>	0.688 *	0.220 <sup>ns</sup>
September, RH%	0.306 <sup>ns</sup>	0.496 <sup>ns</sup>	−0.988 **	−0.998 **	0.975 **	−0.873 **	0.985 **	−0.462 <sup>ns</sup>

Note: TAA—total antioxidant activity; TPC—total phenol content; ACC<sub>HPLC</sub>—total anthocyanin content determined by chromatographically; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside; ns—non-significant correlation coefficient; significant at  $p \leq 0.05$  \*, and  $0.01$  \*\*; SAT—sum of active temperatures ( $\geq 10$  °C); BBCH—phenological growth stage identification scale; SAT BBCH 71–79—active temperatures of June, July; SAT BBCH 81–89—active temperatures of August, September; HI—Heliothermal Index; RF—radiation flux ( $W m^{-2}$ ); frost-free period—sum of days with temperature above 0 °C; precipitation—sum of precipitation (mm); RH—relative air humidity (%).

The principal component analysis (PCA) showed that the first principal component (PC1) explained 46% of the total variance in the data, and the second principal component (PC2) explained 24% (Figure 2). The PC1 and PC2 explained 70% of the variance in the data for both Figure 2a,b. The most important determinants of the PC1 were weather-related parameters such as frost free period, HI, SAT, SAT BBCH81–89, SAT BBCH 71–79, RH% Sept and precipitation (Figure 2a). Among grape quality characteristics, Dp, Mv and Pt were the most important determinants of PC1. PC2 was primarily determined by ACC, TPC, TAA, Cy, Pn, and titratable acids content (TAC). It was clearly seen in the PCA map that all polyphenols, especially Dp, Mv, and Pt, were situated in the same area with frost free period, HI, SAT (BBCH 71–79, BBCH 81–89, year) and RF (August, September). Among experimental years, the year 2018 was clearly situated in the same area (Figure 2b). The year 2017 was distinguished in the opposite site, characterized by high precipitation and RH, causing high TAC of fruits. Among cultivars, ‘Rondo’ was clearly distinguished in the PCA map, situated in the same area with high values of polyphenols and TAA. Other cultivars did not have clear distinction in PCA map.



**Figure 2.** Principal component analysis (PCA) of the structure of biochemical and berry parameters in relation to the cultivar and climatic conditions of experimental years: (a) biochemical and berry parameters in relation to the climatic parameters; (b) cultivar in relation to the experimental year. BW—berry weight; NBC—berries per cluster; CW—cluster weight; SSC—soluble solids content; TAC—titratable acids content; TAA—antioxidant activity; TPC—total phenol content; ACC—total anthocyanin content determined spectrophotometrically; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside; SAT—sum of active temperatures ( $\geq 10$  °C); BBCH—phenological growth stage identification scale; SAT BBCH 71–79—active temperatures of June, July; SAT BBCH 81–89—active temperatures of August, September; HI—Heliothermal Index; RF—radiation flux ( $W m^2$ ); FFP—frost-free period, sum of days with temperature above 0 °C; Prec—sum of precipitation (mm); RH—relative air humidity (%).

## 4. Discussion

### 4.1. The Effect of Year and Cultivar on Phenolic Compounds

In this 9-year experiment, a high variability in TPC and  $ACC_{spec}$  of hybrid grapevine fruits was found as shown in Table 4. Cultivar, year and their interaction significantly affected the contents of these parameters. In addition, correlation and PCA analyses indicated that climatic conditions had an impact on the phenolic compounds in grapes. Warmer and longer vegetation period increased polyphenols content, as seen in years 2018 and more precipitation and higher RH decreased it. The effect was cultivar dependent and ‘Rondo’ differed significantly from the others. TPC in ‘Hasansky Sladky’ and ‘Zilga’ had no correlation with temperature-related parameters, but TPC in ‘Rondo’ had a correlation with climatic parameters. All the experimental cultivars had a correlation between  $ACC_{HPLC}$  and climatic parameters. It may be related to the cultivars’ sensitivity to temperature changes. Experimental cultivars had different cluster properties and this could cause variation between cultivars [24]. Berries of ‘Rondo’ and ‘Zilga’ are larger and more tightly arranged in the clusters than those of ‘Hasansky Sladky’. Additionally, in warmer climates, accumulation of phenolic compounds in *Vitis vinifera* grapes was influenced by environmental factors [5], cultivar [4,6], and *terroir* [7]. The age of the vines also contributed to the years-on-year difference. The vines were three years old at the beginning of the trial, but by the end of the experiment the vines were 11 years old. As the vines grew older, the trunk thickness and the shoots growth intensity changed and that could have affected the results. It is important for vine grower and wine producer to determine the potential of their cultivar of choice for growing.

There was a significant effect of cultivar properties and interaction between year and cultivar on TAA—‘Rondo’ had the highest TAA as shown in Table 5. It has been found that cultivars

with higher TPC had increased TAA as well [8,9]. This was also confirmed in our experiment. In the present study, the content of anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside) in all tested hybrid grapes depended on the cultivar and growth year as shown in Table 5 and Figure 2. The correlation analyses also indicated that climatic conditions had a correlation with individual anthocyanins and the effect depended on cultivar. Similar results are reported in an experiment made in warmer climate with *Vitis vinifera* cultivars [11], which refers that anthocyanin composition depends on the genetic background of *Vitis* species [10]. Still, the differences in anthocyanin composition are related to the cultivar responses to temperature as well [1]. In all experimental cultivars, Dp, Pt and Mv had a positive relationship with temperature-related parameters and a negative one with precipitation. Relationship of Cy and Pn depended on the cultivar properties. The order of occurrence of individual anthocyanins in the field grown grapes varied among years. For example, in 'Rondo' it was: Dp > Pn > Mv > Cy > Pt in 2016, Pn > Dp > Mv > Cy > Pt in 2017, and Dp > Mv > Pt > Pn > Cy in 2018. Anthocyanins give different colors: Cy—crimson, Pn—magenta, Dp—mauve, Pt and Mv—purple. Dp was the dominant anthocyanin in warmer years and Pn in cooler years. In this experiment, 'Rondo' showed higher potential of phenolic compounds.

#### 4.2. The Effect of Viticultural Practice

The experiment with 'Rondo' showed significant effect of the viticultural practice, year and their interaction on the anthocyanin content as seen in Table 6. The abundance of different individual anthocyanins varied between the field and tunnel grown grapes in two years out of three. In other experiments, different viticultural practices have been shown to affect anthocyanin profile [12,13], as was also confirmed in our experiments. Therefore, it can be concluded that the shade of grape color from the hybrid grapes in a very cool climate conditions vary from year to year and depend on the order of the occurrence of individual anthocyanins. The color of the wines depends in a large extent of the anthocyanins content and distribution. Color is an important factor for evaluating the quality of red wine and is one of the most important factors for consumers when choosing a wine. PCA analysis showed that the technological maturity of grapes also have influenced their phenolic compounds. The technological maturity parameters of experimental cultivars at harvest varied significantly between years and sometimes did not reach the recommended technological maturity for wine making.

Viticultural practice had a significant effect on ACC<sub>HPLC</sub> in 'Rondo' as shown in Table 6. In 2016 and 2018, ACC<sub>HPLC</sub> and individual anthocyanins in 'Rondo' were significantly higher in the field cultivated grapes, but in 2017 in the tunnel grown grapes. The contents were significantly influenced by the weather conditions of the experimental years. In 2017, the vegetation period was exceptionally cold and rainy (SAT 1981 °C, HI 888, and precipitation 497 mm). In cooler and rainier years, growing grapes in the tunnel promoted their maturation. In 2016 and 2018, the vegetation period was longer compared to the average, and warmer as well. The year 2018 was exceptionally warm (SAT 2660 °C, HI 1532, and frost-free period 180 days). Elevated temperatures during ripening may reduce the accumulation of anthocyanins and could partly degrade the previously synthesized compounds [14,15]. At *veraison* the temperatures in the tunnel are higher, and day and night temperature fluctuations are greater (min 5 to 6 °C and max 35 to 40 °C) [32]. The differences in growing conditions between the tunnel and the field could have caused variation in compound bud vitality: whether shoots developed from a larger central primary bud, from smaller secondary buds, or from both at the same time. In the field, when the growing season is cooler, the vine primary bud may remain less cold hardy. The second problem is spring frosts. When the primary bud is damaged in cool spring, the smaller secondary or tertiary bud will break, which will greatly affect yield formation.

## 5. Conclusions

During nine years, significant variability of TPC and ACC in hybrid grapevine fruits was found. 'Rondo' had a higher content of total polyphenols and anthocyanins in most of the experimental

years. Variability depended on cultivar and was affected by the climatic parameters of the experimental years. Additionally, the content of individual anthocyanins was affected by the cultivar properties and the climatic parameters. In every year, most abundant individual anthocyanins were malvidin-3-O-glycoside in ‘Hasansky Sladky’ and delphinidin-3-O-glycoside in ‘Zilga’. In ‘Rondo’ grapes, anthocyanin contents varied from year to year and depended on cultivation site (polytunnel/field). TAA depended on cultivar and it was highest in ‘Rondo’ every year, therefore it has potential to produce wines rich in antioxidants. Growing ‘Rondo’ in a high polyethylene tunnel increased the total anthocyanin content during the cooler and rainier year, but decreased it in the warmer year. Individual anthocyanins showed the same tendency. As ‘Rondo’ is not winter hardy in the Estonian climatic conditions, for that reason it is recommended to be grown in a high polyethylene tunnel.

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