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# Genotype and Crop Year Effects on Fruit Quality and Phenolic Composition of 35 Blueberry Cultivars

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**Abstract**

Fruit quality and phenolic composition of blueberry (*Vaccinium* spp.) are cultivar-dependent, yet multi-year characterizations remain limited. The effects of genotype (G), crop year (Y) and their interaction (G × Y) were assessed on pomological traits (fruit weight and length, CIELab color coordinates, soluble solids and titratable acidity) and on the phytochemical profile (total phenolic, anthocyanin and flavan contents and antioxidant activity) of 35 blueberry cultivars of northern highbush, southern highbush, and rabbiteye type, grown under common management in central Italy over three crop years (2020–2022). Individual phenolic compounds were quantified by LC–MS. Genotype was the main source of variation for most pomological traits, while chemical profile (soluble solids content, titratable acidity, total phenolic content, total monomeric anthocyanins and antioxidant activity) was strongly influenced by Y, whereas flavan content was also mainly influenced by Y; the G × Y interaction was significant for all traits but accounted for smaller mean squares. Total phenolic content ranged from 191 to 544 mg GAE 100 $g^{-1}$ FW, total flavan content from 92 to 797 mg CAE 100 $g^{-1}$ FW, and chlorogenic acid from 845 to 3277 mg $kg^{-1}$ FW (Camellia and Ochlockonee, respectively), with rabbiteye cultivars ranking highest. Powder Blue, Ochlockonee, Vernon, Hortblue Petite Rubel and Nui showed the highest phenolic and flavan contents.

**Keywords:** vaccinium; phenolic compounds; pomological traits; antioxidant; LC–MS; genotype × environment interaction

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## 1. Introduction

*Vaccinium corymbosum* L., commonly known as highbush blueberry, is a member of the *Ericaceae* family. The species is native to North America and is now widely cultivated and marketed in the temperate regions of both North America and Europe [1]. According to the FAOSTAT data platform, the global cultivated area was more than 197 thousand ha, and the total production was 1.39 million tonnes in 2024 [2]. Europe accounted for 204,167.7 t, the Americas for 1,098,930.7 t, and Africa for 57,382.0 t. Italy ranks 12th worldwide, with an annual production of 10.3 thousand tonnes, corresponding to 5.1% of the 2024 European production [2].

Blueberries are consumed both as fresh fruit and in their processed forms, such as spreads, jams, syrups, wines, teas, and dairy products. Beyond their organoleptic characteristics, berry fruits are notably rich in bioactive compounds that may provide health benefits when included in the diet [3]. In fact, they are rich sources of polyphenols, and blueberry by-products obtained from fruit processing have been characterized for their phenolic composition and proposed as functional ingredients [4].

Beyond their well-known health benefits, the quality of blueberries is increasingly linked to their levels of phenolic compounds and antioxidant activity [5]. Phenolic molecules are widely distributed across plant species, play central roles in plant defense against biotic and abiotic stresses, and contribute to the color, flavor, and health-promoting attributes of plant-derived foods [6]. They include phenolic acids, flavonoids and tannins; flavonoids alone comprise more than 8000 identified structures, subdivided into anthocyanins, flavan-3-ols and flavonols [7], while chlorogenic acid is the main phenolic acid of blueberry fruit [8]. Reported values of phenolic content in blueberry fruit differ considerably among studies, and these differences have been related to the analytical method applied, the tissue analyzed, the genotype and the growing conditions [9]. Genotype has been reported as a significant source of variation for most fruit quality traits, while significant genotype × year effects indicate that cultivar ranking is not conserved across sites and seasons [10,11]. Temperature and solar radiation are the environmental variables most frequently associated with phenolic accumulation in Vaccinium fruit, acting on the flavonoid pathway both directly and through the rate of fruit development [12,13], and cultivation system and irrigation regime contribute further [14]. Multi-year characterization of a single germplasm collection grown under common agronomic management is therefore required in order to separate the genotypic signal from interannual climatic variation [15].

In parallel with consumer acceptance, fruit breeders and the food industry are moving towards the development of highly nutritious products [16]. A wide range of cultivars differing in ripening time and fruit composition is therefore of considerable market relevance, since it extends the period during which fresh fruit is available to consumers.

Accordingly, the aim of the present study was to assess the influence of genotype and climatic conditions on the main pomological traits [i.e., color, size, weight (W), titratable acidity (TA) and soluble solids content (SSC)] and on the nutraceutical potential [i.e., antioxidant activity (AA), total phenolic content (TPC), total flavan content (FLC) and total monomeric anthocyanins (TMA)] of 35 *blueberry* cultivars evaluated over three consecutive years, in order to identify the genotypes best suited to the market on the basis of their phytochemical profiles.

## 2. Materials and Methods

### 2.1. Plant Materials

Thirty-five blueberry cultivars of northern highbush and southern highbush (*Vaccinium corymbosum* L. and its interspecific hybrids) and rabbiteye (*Vaccinium virgatum* Aiton) types (Table 1) grown in the experimental field of Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA)—Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura (Central Italy, lat. 41.8000° N, 12.5690° E, alt. 86 m a.s.l.) were investigated. The same agronomic techniques (pest control, irrigation, fertilization, etc.) were applied for all cultivars. Fruits were harvested at the same ripening stage (commercial maturity).

Specifically, three individual sets of samples (about 1.5 kg each), obtained from five different plants per genotype, were randomly collected. Three biological replicates ($n = 3$) were analyzed for each genotype in each of three consecutive years (2020–2022), and two analytical laboratory replicates were performed on each biological replicate; their mean value was used for statistical analyses. All quality trait analyses were carried out

on fresh fruits. Before phytochemical traits (TPC, FLC and TMA) and antioxidant activity determinations, the samples were stored at −80 °C.

**Table 1.** Genotype name, type (N = northern highbush; S = southern highbush; R = rabbiteye), breeder/distributor, ripening time under the climatic conditions of central Italy (early: before 5 June; intermediate: 6–19 June; late: after 20 June) and abbreviation of the 35 blueberry genotypes.

| Genotypes | Type | Breeder | Ripening Time | Abbreviation |
|---|---|---|---|---|
| Aurora | N | Michigan State University | Intermediate | AUR |
| Berkeley | N | N/A | Intermediate | BER |
| Blue Ribbon | N | Fall Creek Genetics | Early | BRI |
| Bluecrop | N | University of New Jersey | Intermediate | BCR |
| Blueray | N | N/A | Intermediate | BLR |
| Brigitta Blue | N | Victoria Department of Agriculture | Late | BGB |
| Camellia | S | University of Georgia | Early | CAM |
| Chandler | N | USDA-ARS | Intermediate | CHA |
| Cipria | N | Institute For Plant and Food Research (New Zealand) | Early | CIP |
| Cooper | S | N/A | Early | COO |
| Cosmopolitan | N | Institute For Plant and Food Research (New Zealand) | Early | COS |
| Coville | N | University of New Jersey | Intermediate | COV |
| Darrow | N | USDA-ARS | Intermediate | DAR |
| Draper | N | Michigan State University | Intermediate | DRA |
| Duke | N | University of New Jersey | Intermediate | DUK |
| Early Blue | N | N/A | Early | EBL |
| Elizabeth | N | University of New Jersey | Intermediate | ELI |
| Elliot | N | USDA-ARS | Late | ELL |
| Hortblue Petite | N | Institute For Plant and Food Research (New Zealand) | Early | HBP |
| Hortblue Poppins | N | Institute For Plant and Food Research (New Zealand) | Early | HPO |
| Legacy | S | University of New Jersey | Intermediate | LEG |
| Liberty | N | Michigan State University | Late | LIB |
| Nui | N | Plant and Food Research (New Zealand) | Intermediate | NUI |
| Ochlockonee | R | University of Georgia | Intermediate | OCH |
| Ozark Blue | S | Moore James Norman/Clark John Reuben (Arkansas) | Intermediate | OZB |
| Patriot | N | USDA-ARS | Intermediate | PAT |
| Pink Lemonade | R | N/A | Intermediate | PLE |
| Powder Blue | R | N/A | Intermediate | PWB |
| Rebel | S | University of Georgia | Early | REB |
| Roxy Blue | N | Institute For Plant and Food Research (New Zealand) | Early | RXB |
| Rubel | N | N/A | Intermediate | RUB |
| Spartan | N | USDA-ARS | Early | SPA |
| Sunshine Blue | S | N/A | Intermediate | SUN |
| Toro | N | N/A | Intermediate | TOR |
| Vernon | R | University of Georgia | Late | VER |

Modified from De Salvador et al. [17]. Pink Lemonade is a hexaploid USDA germplasm release derived for one half from *Vaccinium virgatum* (rabbiteye) and for one half from synthetic hexaploid highbush-type germplasm, and is reported here within the rabbiteye group.

### 2.2. Fruit Pomological Traits Evaluation

Pomological traits [i.e., weight, length, width, thickness and skin color (CIELab coordinates L, a*, b*)] were determined on 20 individual fresh fruits for each biological replicate, as previously reported by Ceccarelli et al. [18]. Specifically, a digital caliper (maximum

permissible error ± 0.05 mm) was used to determine the berry dimensions (length, width, thickness) expressed in mm. Ground skin color, on both external opposite fruit sides, was evaluated using the CIELab color space coordinates, obtained with a tristimulus colorimeter (Chroma Meter CR-400; Minolta, Milan, Italy), equipped with a D65 illuminant. The results were expressed as the mean of the two opposite fruit sides for the color coordinates: L (lightness, from 0 = black to 100 = white), a* [from green (−) to red (+)], and b* [from blue (−) to yellow (+)]. Then, the whole fruits were weighed, and titratable acidity (TA) and soluble solids content (SSC) were determined on homogenized whole fresh fruits (about 200 g per genotype for each biological replicate), according to Ceccarelli et al. [18].

The method for titratable acidity was based on titration of the acids present in the juice with sodium hydroxide (0.1 mol $L^{-1}$) using an automatic titration system (785 DPM Titrino, Metrohm Ltd., Herisau, Switzerland). Results were expressed as mEq $L^{-1}$ and converted into grams of citric acid equivalents per 100 g of fresh weight using the equivalent weight of citric acid (192.12/3 = 64.04 mg $mEq^{-1}$) and assuming a juice density of 1 g $mL^{-1}$, that is, TA (g 100 $g^{-1}$ FW) = TA (mEq $L^{-1}$) × 0.006404. The SSC:TA ratio was calculated as SSC (g 100 $g^{-1}$ FW) divided by TA (g 100 $g^{-1}$ FW) and is therefore dimensionless.

The SSC was evaluated by a digital refractometer (Refracto 30 PX, Mettler Toledo, Milan, Italy). Measurements were conducted at 20 °C, and results were expressed as grams per 100 g of fresh weight (g 100 $g^{-1}$ FW).

### 2.3. Chemicals

All reagents used were of analytical spectrophotometric grade (Carlo Erba, Rome, Italy). Folin–Ciocalteu reagent, gallic acid, catechin, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), vanillin and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Milan, Italy). Cyanidin 3-O-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside standards used for identification and quantification purposes with HPLC were purchased from Extrasynthese (Genay, France) and Sigma-Aldrich (Milan, Italy). Organic solvents used for chromatography were of HPLC ultragradient grade (Sigma-Aldrich, Milan, Italy), whereas ultrapure water was obtained by a Milli-Q system (Millipore, Milan, Italy). The 0.22 µm pore size membrane filters were purchased from Pall (Pall Corporation, Ann Arbor, MI, USA) and used for the filtration of samples.

### 2.4. Extraction for Colorimetric Determination of Secondary Metabolites and Antioxidant Activity

Secondary metabolite extraction was carried out on 5 g of thawed samples. Briefly, each sample was homogenized with a blender (Ultra-Turrax T25, IKA Labortechnik, Staufen, Germany) in 25 mL of methanol solution (methanol/water 70/30 v/v added with 5 mM HCl) and subsequently incubated in a thermostatic bath at 37 °C for 2 h, then centrifuged (centrifuge mod. 4239R, ALC International—Milan, Italy) at 8000 g for 15 min at 5 °C. The supernatant was collected, and the pellet was re-extracted with 20 mL of the same solvent in the water bath for 30 min and centrifuged again under the same conditions. The two supernatants were combined and brought to a final volume of 50 mL with the extraction solvent. The procedure was adapted from Ceccarelli et al. [18]. The extract was used to determine TPC, FLC, TMA, and AA.

### 2.5. Colorimetric Determination of Secondary Metabolites and Antiradical Capacity

TPC was determined by the Folin–Ciocalteu method according to Ceccarelli et al. [18] and Lolletti et al. [19], and results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 $g^{-1}$ FW). FLC was determined by the vanillin assay according to Sun, Ricardo-da-Silva and Spranger [20], and results were expressed as milligrams of catechin equivalents per 100 g of fresh weight (mg CAE 100 $g^{-1}$ FW).

TMA was determined by the pH differential method [21], and results were expressed as milligrams of cyanidin 3-O-glucoside equivalents per 100 g of fresh weight (mg C3GE 100 $g^{-1}$ FW).

Finally, AA was determined by the DPPH assay as described by Braca et al. [22] and modified according to Ceccarelli et al. [18].

Results were expressed as micrograms of Trolox equivalents (TE) per milligram of fresh weight (µg TE $mg^{-1}$ FW). All determinations were performed in triplicate.

### 2.6. Extraction for Phenolic Compounds Profiling

Phenolic compounds were extracted according to the method of Ceccarelli et al. [23]. Briefly, the frozen fruit material (about 5 g) was homogenized by a blender (Ultra-Turrax T25, IKA Labortechnik, Staufen, Germany) with 10 mL of extraction solution (methanol/water 80/20 $v/v$) containing 2 mM sodium fluoride and hydrochloric acid (5 mM). Homogenates were centrifuged at 8000 × $g$ and 5 °C for 15 min, and the supernatant was filtered through a 0.2 µm filter (Acrodisc®, Pall Corporation) and stored at −80 °C until the LC-MS analysis.

### 2.7. Chromatographic Evaluation of the Phenolic Profile

Chromatographic analyses were performed using an Agilent 1290 Infinity Binary LC with a UV–vis photodiode array detector (DAD G4212A), coupled with an Agilent 6530 Accurate Mass Quadrupole Time-of-Flight, equipped with a dual spray ESI source (Agilent, Milan, Italy) as previously described by Ceccarelli et al. [23]. Briefly, Kinetex C18 (150 × 2.1 mm i.d., 1.7 µm) from Phenomenex (Torrance, CA, USA), as well as acidified water (A) and acetonitrile (B) solvents, both containing 0.1% ($v/v$) formic acid, were used. The applied chromatographic conditions were: flow rate 0.3 mL $min^{-1}$; injected volume 1 µL; isocratic conditions at 5% B from 0 to 10 min; linear gradient from 5% to 17% B in 3 min (10–13 min); isocratic conditions at 17% B from 13 to 18 min; linear gradients from 17% to 45% B in 7 min (18–25 min), from 45% to 65% B in 5 min (25–30 min) and from 65% to 90% B in 4 min (30–34 min); isocratic conditions at 90% B from 34 to 44 min to wash the column. Then the initial conditions (5% B) were restored, and the column was re-equilibrated for 5 min before the next injection. As previously reported by Ceccarelli et al. [23], electrospray mass spectrometric analysis was performed in negative mode, and nitrogen was used as nebulizer gas at a pressure of 414 kPa (60 psi), with a flow of 8 L $min^{-1}$. Applied chromatographic conditions were: a temperature of 350 °C with a flow of 8 L $min^{-1}$; capillary voltage was set at 2800 V; and fragmentor voltage was fixed at 175 V. All data were acquired in centroid mode.

The phenolic compounds in blueberry fruit extracts were identified and quantified by calibration curves of certified standards, using Mass Hunter Qualitative and Quantitative Analysis software (ver. B.05.00), and concentrations were expressed as mg $kg^{-1}$ FW. The compound class, molecular formula, measured m/z, ionization mode, and retention time of each identified compound are reported in Table S1 of the Supplementary Materials. Chlorogenic acid, (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, quercetin 3-O-rutinoside, quercetin 3-O-glucoside, cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside and peonidin 3-O-rutinoside (Extrasynthese, Genay, France) were used as external standards for the identification and quantification of single compounds. The quantification method had been previously validated in terms of sensitivity, repeatability, and linearity: Limits of detection (LODs) ranged from 6.4 to 635 µg $kg^{-1}$, repeatability showed relative standard deviation (RSD) values below 15%, and calibration curves demonstrated excellent linearity ($R^2 > 0.99$).

Neochlorogenic, cryptochlorogenic, and p-coumaroylquinic acids, for which authentic standards were not available, were quantified as chlorogenic acid equivalents and identified

by comparing their retention times and MS and MS/MS spectra with those reported in the literature [24]. Kaempferol rutinoside was quantified as quercetin 3-O-rutinoside equivalents; the peonidin glycosides were quantified as peonidin 3-O-rutinoside equivalents, and the delphinidin, petunidin, malvidin and pelargonidin glycosides as cyanidin 3-O-glucoside equivalents, all identified in negative ionization mode according to J. Sun et al. [25].

### 2.8. Statistical Analysis

Prior to the analysis of variance, data were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. The assumptions required for ANOVA were met for all variables analyzed.

Analysis of variance, Duncan's test, Pearson's correlation coefficients ($r$), and least significant difference (LSD) were used to evaluate statistically significant differences among genotypes. Mean comparisons were performed using Duncan's multiple range test at $p \leq 0.05$. The experimental unit was the biological replicate, defined as about 1.5 kg of fruit collected from the same group of five plants per genotype; the two analytical determinations carried out on each biological replicate were averaged before the statistical analysis, so that each genotype was represented by three biological replicates in each crop year, giving 315 observations. The same three groups of plants were sampled in each of the three crop years, and the data were therefore analyzed by two-way repeated measures ANOVA, with genotype (35 levels) and crop year (3 levels) as fixed factors and the replicate groups as repeated subjects; each effect was tested against its own error term .

Seasonal associations between fruit traits and the climatic conditions of the ripening period were examined descriptively by comparing the annual means of the traits with the corresponding meteorological variables of each crop year. No constrained ordination was applied because the climatic variables take only three distinct values, one per crop year, which does not provide the independent environmental replication required to estimate and test the effects of six predictors. The traits considered were W, H, the CIELab coordinates (L, a*, b*), SSC, TA, TPC, FLC, TMA and AA, and the meteorological variables were those of the ripening period (TMIN IV, TMAX IV, TMEAN IV, R IV, RH IV and SR IV). All other analyses were performed with PAST (version 4.03 [26]).

## 3. Results and Discussion

### 3.1. Agroclimatic Conditions

Climatic conditions differed among the three crop years (2019/20, 2020/21 and 2021/22, corresponding to the 2020, 2021 and 2022 harvests; Figure 1); phases I–IV correspond to the four quarters of the crop year defined in the caption of Figure 1. Rainfall varied substantially among seasons, particularly during phase II, with cumulative precipitation reaching 310 mm in 2020/21, compared with 220 mm in 2019/20 and 155 mm in 2021/22. Lower rainfall amounts were recorded during phases III and IV, ranging from 47 to 130 mm. During phase I, precipitation decreased progressively from 180 mm in 2019/20 to 140 mm in 2021/22.

Mean solar radiation was highest in phase IV, increasing from 680 W $m^{-2}$ in 2019/20 to 780 W $m^{-2}$ in 2021/22, and lowest in phase II (140–230 W $m^{-2}$), with intermediate and more stable values in phases I (360–450 W $m^{-2}$) and III (360–400 W $m^{-2}$).

Minimum temperatures differed among seasons mainly in phase I (8.6 °C in 2020/21 to 15.2 °C in 2021/22) and fell below 0 °C in phases II and III in all seasons (−2.1 to −1.0 °C). Maximum temperatures remained high in phase I (37.6–38.6 °C) and increased in phase IV from 37.8 to 40.0 °C over the three seasons, whereas in phases II and III they were lower and more variable, with the lowest values in 2021/22 (9.4 and 10.8 °C).

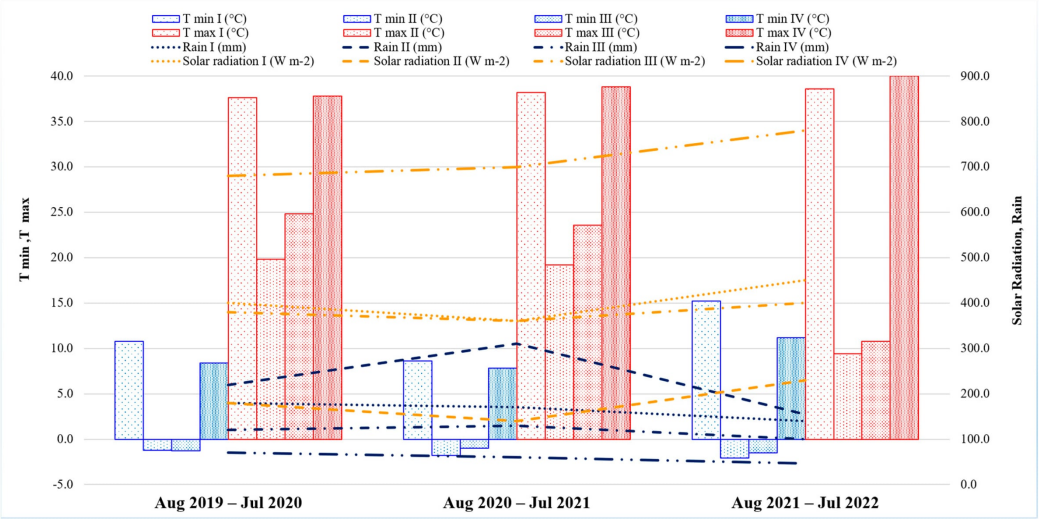



**Figure 1.** Means of the meteorological data [i.e., minimum temperatures (°C), maximum temperatures (°C), rainfall (mm) and mean solar radiation (W/m$^2$)] grouped by quarter of the crop year (phase I: August–October; phase II: November–January; phase III: February–April; phase IV: May–July) for the three crop years (2019/20, 2020/21 and 2021/22). Legend: T min, T max, Rain, and Solar radiation indicate minimum temperature, maximum temperature, rainfall, and solar radiation, respectively; the Roman numerals I to IV identify the quarter of the crop year, as defined above.

Compared with the previous seasons, 2021/22 was characterized by lower precipitation during all phases, higher solar radiation during phases I, II and IV, and higher minimum and maximum temperatures during phases I and IV. Conversely, 2020/21 recorded the highest rainfall during phase II and the lowest solar radiation during phases I and II.

### *3.2. Pomological Attributes*

A two-factor ANOVA was performed to assess the effects of genotype (G), crop year (Y), and their interaction (G × Y) on the pomological and chemical traits of 35 blueberry genotypes evaluated over three consecutive years (Table 2). Blueberry quality is affected by several factors, including cultivar, ploidy level (2x, 4x or 6x), environmental conditions, soil characteristics, water availability, crop management practices, and fruit maturity stage [27].

In the present study, genotype significantly affected all measured variables ($p < 0.001$), namely fruit weight, fruit length, color coordinates (L, a* and b*), soluble solids content (SSC) and titratable acidity (TA), and SSC:TA ratio. The large genotype mean squares indicate substantial genetic variability among the evaluated accessions for both pomological and compositional characteristics. Similar findings were reported by Pavliuk et al. [28], who evaluated the phenological and pomological characteristics of 32 blueberry cultivars over a five-year period (2020–2024). Their results revealed marked differences among cultivars, confirming the major role of genetic background in determining blueberry performance and fruit characteristics.

Crop year also exerted a considerable influence on several traits. Fruit weight, SSC and TA were significantly affected by year ($p < 0.001$) and lightness at $p < 0.05$, whereas fruit length, the color coordinates a* and b* and the SSC:TA ratio were not. These results agree with those reported by Gilbert et al. [29], who observed considerable variability among blueberry cultivars and emphasized the role of environmental conditions in determining key compositional traits, particularly SSC and TA.

**Table 2.** Pomological and chemical traits of the 35 blueberry genotypes over three crop years: (**A**) two-way repeated measures analysis of variance (ANOVA), reporting the mean squares for the effects of genotype (G), crop year (Y) and their interaction (G × Y); (**B**) mean values ± standard deviation, for genotypes and crop years, of fruit weight (W, g), fruit length (H, mm), CIELab color coordinates (L, a*, b*), soluble solids content (SSC, g 100 $g^{-1}$ FW), titratable acidity (TA, mEq $L^{-1}$) and SSC:TA (ratio of SSC to TA expressed as g citric acid equivalents 100 $g^{-1}$ FW).

(**A**)

| SOURCE | DF | W | H | L | a * | b * | SSC | TA | SSC:TA |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes (G) | 34 | **1.774 ***** | **127.8 ***** | **138.1 ***** | **125.5 ***** | **84.3 ***** | 14.1 *** | 20,506 *** | **910.9 ***** |
| Crop Years (Y) | 2 | 0.574 *** | 16.5 *** | 19.4 ns | 0.878 ns | 0.950 ns | **124.6 ***** | **25,367 ***** | < 0.001 ns |
| G × Y | 68 | 0.0014 *** | 0.03 ns | 4.58 ns | 1.48 ns | 0.692 ns | 1.451 *** | 488 *** | < 0.001 ns |
| Error (G × subject) | 68 | 0.00116 | 0.50 | 3.22 | 2.02 | 1.00 | 0.0124 | 20.2 | 0.051 |
| Error (Y × subject) | 4 | 0.000502 | – | 11.65 | 4.37 | 0.740 | 0.109 | 22.0 | < 0.001 |
| Error (G × Y × subject) | 136 | 0.00000092 | – | 5.05 | 2.01 | 0.994 | 0.00127 | 0.411 | < 0.001 |
| Total | 314 | | | | | | | | |

(**B**)

| | | W | H | L | a * | b * | SSC | TA | SSC:TA |
|---|---|---|---|---|---|---|---|---|---|
| GENOTYPES | Aurora | 1.51 ± 0.09 cd | 14.1 ± 0.7 e | 36.84 ± 2.37 | 0.95 ± 1.05 c | -5.25 ± 0.91 cd | 11.2 ± 0.6 b | 219 ± 12 a | 8.0 |
| | Berkeley | 1.72 ± 0.10 c | 14.9 ± 0.7 e | 42.03 ± 2.50 b | 0.01 ± 0.40 d | −5.99 ± 0.36 cd | 9.5 ± 1.2 bc | 74 ± 10 d | 20.0 |
| | Blue Ribbon | 1.91 ± 0.12 b | 15.4 ± 0.8 de | 39.80 ± 2.23 b | 0.47 ± 0.50 d | −5.63 ± 0.94 cd | 11.1 ± 0.7 b | 141 ± 9 bc | 12.3 |
| | Bluecrop | 1.21 ± 0.07 | 13.5 ± 0.7 | 41.49 ± 1.63 b | 2.16 ± 1.11 b | −6.93 ± 1.04 d | 10.6 ± 1.3 b | 199 ± 11 ab | 8.3 |
| | Blueray | 1.90 ± 0.11 b | 15.4 ± 0.8 de | 35.67 ± 1.89 | 1.18 ± 0.72 c | −4.91 ± 0.62 c | 10.4 ± 1.4 b | 171 ± 23 b | 9.5 |
| | Brigitta Blue | 1.89 ± 0.11 b | 14.9 ± 0.7 de | 37.60 ± 1.63 | 1.25 ± 0.69 c | −5.28 ± 0.48 c | 10.8 ± 1.3 b | 114 ± 13 c | 14.8 |
| | Camellia | 1.97 ± 0.12 b | 15.2 ± 0.8 de | 41.39 ± 2.47 b | 1.45 ± 1.03 b | −6.81 ± 0.92 d | 9.8 ± 1.3 bc | 122 ± 16 c | 12.5 |
| | Chandler | 1.77 ± 0.11 c | 14.6 ± 0.7 e | 35.59 ± 3.08 | 0.42 ± 0.50 d | −5.02 ± 1.33 c | 9.7 ± 1.9 bc | 102 ± 20 c | 14.8 |
| | Cipria | 1.16 ± 0.07 e | 12.5 ± 0.6 f | 38.43 ± 2.30 bc | 2.18 ± 1.45 b | −5.39 ± 0.80 cd | 8.9 ± 1.4 c | 135 ± 21 bc | 10.3 |
| | Cooper | 1.72 ± 0.08 c | 15.5 ± 2.2 de | 36.85 ± 1.91 | 2.15 ± 2.18 b | −5.74 ± 1.29 c | 11.1 ± 0.6 b | 199 ± 11 ab | 8.7 |
| | Cosmopolitan | 1.31 ± 0.11 de | 15.1 ± 1.1 de | 35.29 ± 1.30 | 1.28 ± 0.87 b | −5.60 ± 0.68 c | 9.3 ± 2.3 bc | 198 ± 49 ab | 7.3 |
| | Coville | 2.04 ± 0.07 b | 9.5 ± 0.6 g | 35.75 ± 1.84 | 0.90 ± 0.78 bc | −4.45 ± 0.72 c | 11.8 ± 1.0 b | 195 ± 17 ab | 9.4 |
| | Darrow | 0.72 ± 0.10 | 19.8 ± 1.0 c | 35.23 ± 0.96 | 1.06 ± 0.76 bc | −5.41 ± 0.47 c | 12.3 ± 1.4 ab | 218 ± 25 a | 8.8 |
| | Draper | 1.59 ± 0.10 cd | 6.9 ± 0.6 h | 39.05 ± 2.76 b | 1.67 ± 1.54 bc | −5.20 ± 1.18 c | 11.5 ± 1.8 ab | 244 ± 39 a | 7.4 |
| | Duke | 1.72 ± 0.08 c | 15.3 ± 0.8 de | 39.63 ± 1.61 b | 2.51 ± 2.03 b | −5.69 ± 1.26 c | 10.4 ± 1.0 b | 153 ± 15 bc | 10.6 |
| | Early Blue | 1.33 ± 0.05 de | 13.2 ± 0.7 f | 30.03 ± 2.20 | 1.68 ± 1.03 bc | −2.12 ± 0.93 c | 9.6 ± 1.3 bc | 172 ± 24 b | 8.7 |
| | Elizabeth | 0.55 ± 0.10 g | 6.1 ± 0.5 h | 33.32 ± 1.89 | 1.03 ± 0.50 c | −5.03 ± 0.71 cd | 9.2 ± 0.8 c | 125 ± 11 c | 11.5 |
| | Elliot | 1.62 ± 0.03 cd | 14.4 ± 0.7 e | 41.44 ± 1.65 b | −0.07 ± 0.35 d | −6.86 ± 0.36 d | 10.2 ± 0.5 b | 171 ± 9 b | 9.3 |
| | Hortblue Petite | 0.56 ± 0.09 | 9.7 ± 0.5 g | 33.61 ± 3.06 | 2.67 ± 1.30 b | −4.71 ± 0.68 c | 10.0 ± 1.4 b | 138 ± 20 bc | 11.3 |
| | Hortblue Poppins | 1.05 ± 0.10 f | 10.6 ± 0.5 g | 34.48 ± 2.01 c | 1.22 ± 1.18 c | −5.12 ± 1.28 cd | 9.5 ± 0.8 c | 108 ± 9 c | 13.7 |
| | Legacy | 1.54 ± 0.09 cd | 10.4 ± 0.5 g | 39.97 ± 1.80 b | 0.87 ± 0.94 cd | −5.63 ± 0.66 c | 12.0 ± 0.7 ab | 155 ± 12 bc | 12.1 |
| | Liberty | 1.42 ± 0.08 d | 15.7 ± 0.8 d | 41.83 ± 2.98 b | 0.21 ± 0.35 de | −7.13 ± 0.55 d | 12.0 ± 0.6 ab | 116 ± 6 c | 16.2 |

**Table 2.** *Cont.*

| | | (**B**) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | W | H | L | a * | b * | SSC | TA | SSC:TA |
| GENOTYPES | Nui | 1.38 ± 0.08 de | 13.1 ± 0.6 f | 38.45 ± 1.45 b | 2.87 ± 1.54 b | −5.33 ± 1.05 cd | 12.0 ± 1.0 ab | 198 ± 17 ab | 9.5 |
| | Ochlockonee | 1.72 ± 0.15 c | 13.2 ± 0.7 f | 36.15 ± 1.89 bc | 1.10 ± 0.96 c | −6.95 ± 0.55 b | 13.6 ± 1.1 a | 107 ± 7 c | 19.8 |
| | Ozark Blue | 2.42 ± 0.11 a | 21.1 ± 1.0 b | 37.35 ± 1.38 bc | 0.83 ± 0.66 c | −5.70 ± 0.56 c | 12.1 ± 1.1 ab | 141 ± 13 bc | 13.4 |
| | Patriot | 1.83 ± 0.07 b | 25.7 ± 1.3 a | 37.87 ± 1.40 bc | 1.16 ± 0.88 c | −5.84 ± 0.83 c | 10.1 ± 0.7 b | 215 ± 15 a | 7.3 |
| | Pink Lemonade | 1.23 ± 0.06 e | 12.7 ± 0.6 f | 52.37 ± 5.43 a | 22.84 ± 4.29 a | 11.82 ± 2.58 a | 12.1 ± 1.2 ab | 29 ± 3 e | 65.2 |
| | Powder Blue | 1.72 ± 0.11 c | 19.9 ± 1.0 c | 37.94 ± 1.72 bc | 0.88 ± 1.41 c | −6.02 ± 0.44 d | 13.5 ± 0.7 a | 135 ± 6 c | 15.6 |
| | Rebel | 1.79 ± 0.11 c | 14.9 ± 0.7 de | 43.96 ± 2.70 b | 1.43 ± 1.21 c | −6.98 ± 1.57 d | 9.7 ± 2.0 bc | 134 ± 27 c | 11.3 |
| | Roxy Blue | 1.75 ± 0.11 c | 15.2 ± 0.8 de | 34.27 ± 1.36 c | 2.89 ± 2.75 b | −5.25 ± 1.18 c | 10.9 ± 1.5 b | 154 ± 21 bc | 11.1 |
| | Rubel | 0.88 ± 0.05 f | 11.4 ± 0.6 fg | 34.16 ± 1.59 c | −0.14 ± 0.61 e | −5.42 ± 0.64 c | 11.1 ± 0.5 b | 100 ± 7 cd | 17.3 |
| | Spartan | 1.60 ± 0.10 cd | 14.5 ± 0.7 e | 35.04 ± 1.92 c | 1.97 ± 1.48 b | −4.54 ± 0.67 c | 9.9 ± 1.3 b | 104 ± 14 cd | 14.9 |
| | Sunshine Blue | 0.91 ± 0.06 f | 11.9 ± 0.6 fg | 35.96 ± 2.11 c | 1.94 ± 1.48 b | −4.51 ± 0.93 c | 10.1 ± 0.8 b | 106 ± 8 cd | 14.9 |
| | Toro | 2.20 ± 0.13 a | 16.1 ± 0.8 d | 40.27 ± 1.18 b | 0.63 ± 0.90 d | −5.32 ± 0.45 c | 10.4 ± 1.3 b | 157 ± 20 bc | 10.3 |
| | Vernon | 1.39 ± 0.08 de | 13.4 ± 0.7 f | 38.88 ± 1.59 b | 0.47 ± 0.34 de | −5.22 ± 0.57 c | 13.6 ± 1.2 a | 72 ± 7 d | 29.5 |
| YEARS | **2020** | 1.44 ± 0.08 b | 13.7 ± 0.7 a | 37.1 ± 1.2 bc | 1.0 ± 0.9 f | −5.5 ± 0.2 cd | 11.7 ± 0.6 a | 228 ± 12 a | 8.0 |
| | **2021** | 1.51 ± 0.08 a | 14.3 ± 0.7 a | 37.1 ± 4.1 bc | 1.5 ± 1.5 bcd | −5.1 ± 1.7 cd | 11.3 ± 0.6 b | 219 ± 11 ab | 8.1 |
| | **2022** | 1.58± 0.08 a | 14.3 ± 0.7 a | 36.3 ± 1.9 bc | 0.3 ± 0.3 de | −5.2 ± 0.7 cd | 10.8 ± 0.6 b | 210 ± 11 b | 8.0 |

Legend: degrees of freedom (DF); values in bold indicate, for each trait, the source of variation with the largest mean square. * significant ($p < 0.05$); ** significant ($p < 0.01$); *** significant ($p < 0.001$); ns, not significant. Different letters within a column indicate significant differences ($p < 0.05$).

The G × Y interaction was significant for fruit weight, SSC and TA, indicating differential genotype responses across the three growing seasons. In contrast, fruit length, the color coordinates, and the SSC:TA ratio showed no significant interaction effect, suggesting a relatively stable expression of these traits across seasons.

Genetic factors therefore represented the main source of variation for most pomological attributes, while the interaction effects observed for SSC and TA indicate that genotype ranking was partly dependent on the growing conditions of each year, which confirms the need for multi-year trials [19,30].

The magnitude of the G × Y mean squares relative to those of the main effects indicates that the ranking of the genotypes for the compositional traits was only partly conserved across seasons. Such behavior is commonly described in perennial fruit crops, where the environment modulates the expression of genetically determined quality traits rather than overriding it [15,30]. It should be noted, however, that the present design, based on three seasons at a single site, allows the interaction to be detected but not partitioned into its environmental components; a formal stability analysis over a wider set of environments would be required to rank the cultivars according to the consistency of their performance.

Pomological and chemical traits varied widely among the 35 genotypes (Table 2B). Fruit weight ranged from 0.55 g in Elizabeth to 2.42 g in Ozark Blue, and fruit length from 6.1 mm in Elizabeth to 25.7 mm in Patriot. Berry size is largely determined by the genetic background, although environmental conditions and cultural practices also contribute to phenotypic variation. The values fall within the ranges previously reported for cultivated blueberries [27].

Significant differences were also observed in skin color. Most genotypes showed the typical bluish coloration, with negative b* and low positive a* values, whereas Pink Lemonade was clearly distinct, with the highest lightness (L* = 52.37), positive b* (11.82), and markedly greater redness (a* = 22.84), consistent with its pink fruit. Among the blue-fruited cultivars, L* ranged from 30.03 in Early Blue to 43.96 in Rebel.

Soluble solids content (SSC) ranged from 8.9 g 100 $g^{-1}$ FW in Cipria to 13.6 g 100 $g^{-1}$ FW in Vernon, with most cultivars between 9.5 and 12.1 g 100 $g^{-1}$ FW, in agreement with De Salvador et al. [17]. Titratable acidity (TA) ranged from 29 mEq $L^{-1}$ in Pink Lemonade to 244 mEq $L^{-1}$ in Draper; among the blue-fruited cultivars, the lowest values were recorded in Vernon (72 mEq $L^{-1}$) and Berkeley (74 mEq $L^{-1}$).

The SSC:TA ratio summarizes these differences in flavor balance. The highest value was recorded in Pink Lemonade (65.2), which combines low acidity with a relatively high sugar content, followed among the blue-fruited genotypes by Vernon (29.5), Berkeley (20.0) and Ochlockonee (19.8); the lowest values (7.3–7.4) were recorded in Cosmopolitan, Patriot and Draper.

The ranges recorded here are comparable to those reported for blueberry cultivars grown under different environments and seasons [27,31], which indicates that the genotype governs the level of these traits while the environment modulates their expression.

Mean berry weight increased progressively from 1.44 g in 2020 to 1.58 g in 2022, whereas SSC decreased from 11.7 to 10.8 g 100 $g^{-1}$ FW and TA from 228 to 210 mEq $L^{-1}$. Differences in fruit length (13.7–14.3 mm) and in the color coordinates were small, although significant for length and lightness (Table 2A).

The year-to-year variation in berry composition was associated with the climatic conditions during fruit development and ripening. The higher berry weight recorded in 2022 coincided with lower precipitation and greater solar radiation, particularly during phases I and IV, and with higher temperatures in the later stages of the season; since the orchard was irrigated, the lower rainfall is unlikely to have limited plant water status, and the response can be attributed mainly to the thermal and radiative components of the season.

The direction of this response deserves comment, because a diurnal temperature increase of about 5 °C applied between fruit set and harvest reduced $CO_2$ assimilation, stomatal conductance and fruit weight in field-grown highbush blueberry, while increasing soluble solids [32]; the temperatures recorded here therefore appear to have remained below the range at which carbon assimilation becomes limiting for fruit growth. The increase in berry weight was accompanied by a decline in SSC and TA. An inverse relationship between berry weight and fruit composition has been reported for blueberry [33], while the decrease in acidity is consistent with the temperature dependence of organic acid catabolism during ripening [34]. Because cultivar ranking for these traits is not necessarily conserved across seasons and sites [10], these interannual differences refer to the specific climatic sequence of the three seasons examined.

### *3.3. Health-Related Compounds and Antiradical Capacity*

A two-factor ANOVA revealed that genotype (G), crop year (Y), and their interaction (G × Y) significantly affected all the bioactive compounds and antioxidant-related traits evaluated, including total phenolic content (TPC), total monomeric anthocyanins (TMA), total flavan content (FLC), and antioxidant activity (AA) (Table 3).

The genotype effect was highly significant ($p < 0.001$) for all variables, which indicates substantial genetic variability in the accumulation of health-promoting compounds and in antioxidant potential. Significant year effects ($p < 0.001$) were detected for TPC, TMA, FLC and AA, with particularly large mean squares for TMA and AA, and the genotype × year interaction was significant for all traits ($p < 0.001$), indicating that the relative performance of the cultivars was not constant across seasons and that multi-year evaluation is required to assess their stability.

Both genetic and environmental factors therefore contribute to the phenolic content and antioxidant potential of blueberry fruit, with the crop years accounting for the largest mean square for TPC, TMA, FLC and AA, while the genotype × year interaction, although always significant, accounted for smaller mean squares with the exception of flavans, which were found to be more strongly influenced by the genotype × year interaction. Comparable results have been reported previously, with phenolic composition and antioxidant capacity varying with cultivar, climatic conditions and their interaction [14,15,35].

**Table 3.** Health-related compounds and antioxidant activity of the 35 blueberry genotypes over three crop years: (**A**) two-way repeated measures analysis of variance (ANOVA), reporting the mean squares for the effects of genotype (G), crop year (Y) and their interaction (G × Y); (**B**) mean values ± standard deviation, for genotypes and crop years, of total phenolic content (TPC, mg GAE 100 $g^{-1}$ FW), total monomeric anthocyanins (TMA, mg C3GE 100 $g^{-1}$ FW), total flavan content (FLC, mg CAE 100 $g^{-1}$ FW) and antioxidant activity (AA, μg TE $mg^{-1}$ FW).

(**A**)

| SOURCE | DF | TPC | TMA | FLC | AA (DPPH) |
|---|---|---|---|---|---|
| Genotypes (G) | 34 | 62,770 *** | 3265 *** | $1.9 \times 10^5$ *** | 0.116 *** |
| Crop Years (Y) | 2 | **94,460 *** ** | **98,120 *** ** | **$2.4 \times 10^6$ *** ** | **0.758 *** ** |
| G × Y | 68 | 13,430 *** | 1471 *** | $5.8 \times 10^4$ *** | 0.086 *** |
| Error (G × subject) | 68 | 268 | 3.47 | 149 | 0.00141 |
| Error (Y × subject) | 4 | 985 | 141 | 219 | 0.0108 |
| Error (G × Y × subject) | 136 | 303 | 3.87 | 118 | 0.000958 |
| Total | 314 | | | | |

**Table 3.** *Cont.*

| | | (**B**) | | | |
|---|---|---|---|---|---|
| | | TPC | TMA | FLC | $AA_{(DPPH)}$ |
| GENOTYPES | Aurora | 338± 64 cd | 69 ± 34 abc | 222± 112 ef | 1.25 ± 0.11 c |
| | Berkeley | 256 ± 49 e | 40± 13 c | 148± 40 fg | 1.29 ± 0.04 c |
| | Blue Ribbon | 301± 51 d | 58± 39 bc | 180 ± 30 f | 1.24 ± 0.18 c |
| | Bluecrop | 302 ± 56 d | 36± 14 e | 278 ± 88 de | 1.64 ± 0.18 a |
| | Blueray | 284± 46 de | 52± 22 c | 198 ± 139 f | 1.51 ± 0.17 ab |
| | Brigitta Blue | 191± 39 f | 42± 15 c | 92 ± 32 g | 1.37 ± 0.17 bc |
| | Camellia | 304± 62 d | 64± 25 abc | 326 ± 129 d | 1.36 ± 0.26 bc |
| | Chandler | 258 ± 69 e | 43 ± 37 de | 290 ± 61 de | 1.40 ± 0.15 b |
| | Cipria | 298 ± 51 cde | 26± 8 e | 200 ± 37 f | 1.66 ± 0.18 a |
| | Cooper | 341± 79 cd | 56± 28 bc | 325 ± 119 d | 1.43 ± 0.11 b |
| | Cosmopolitan | 204 ± 57 f | 57± 34 bc | 97 ± 68 g | 1.48 ± 0.14 b |
| | Coville | 244 ± 36 e | 27± 9 e | 248 ± 33 e | 1.39 ± 0.10 b |
| | Darrow | 246 ± 26 e | 41± 33 c | 206 ± 66 f | 1.29 ± 0.13 c |
| | Draper | 317 ± 49 d | 54 ± 22 bc | 273 ± 105 de | 1.47 ± 0.05 b |
| | Duke | 315 ± 77 d | 82 ± 35 ab | 241 ± 102 e | 1.43 ± 0.21 b |
| | Early Blue | 293 ± 88 cde | 87 ± 29 ab | 153 ± 18 fg | 1.28 ± 0.20 c |
| | Elizabeth | 266 ± 64 e | 60 ± 37 abc | 164 ± 76 fg | 1.38 ± 0.09 bc |
| | Elliot | 336 ± 71 cd | 57± 16 bc | 351 ± 96 cd | 1.35 ± 0.08 bc |
| | Hortblue Petite | 467 ± 89 b | 80 ± 41 ab | 784 ± 288 a | 1.50 ± 0.12 b |
| | Hortblue Poppins | 279 ± 53 e | 70 ± 46 abc | 106 ± 71 g | 1.54 ± 0.13 ab |
| | Legacy | 415 ± 20 bc | 75 ± 43 ab | 255 ± 105 e | 1.52 ± 0.13 b |
| | Liberty | 212 ± 20 f | 48 ± 23 c | 130 ± 65 fg | 1.29 ± 0.23 c |
| | Nui | 290 ± 40 de | 66 ± 47 abc | 299 ± 88 d | 1.69 ± 0.05 a |
| | Ochlockonee | 533 ± 44 a | 71 ± 19 ab | 582 ± 135 b | 1.49 ± 0.02 b |
| | Ozark Blue | 285 ± 63 de | 29 ± 13 e | 227 ± 107 e | 1.29 ± 0.19 c |
| | Patriot | 295 ± 68 de | 58 ± 20 bc | 338 ± 128 d | 1.38 ± 0.15 bc |
| | Pink Lemonade | 258 ± 98 de | 7 ± 4 f | 322 ± 248 d | 1.54 ± 0.20 ab |
| | Powder Blue | 544 ± 202 a | 79± 3 b | 797 ± 181 a | 1.41 ± 0.17 b |
| | Rebel | 347 ± 81 cd | 46 ± 18 c | 404 ± 118 c | 1.49 ± 0.11 b |
| | Roxy Blue | 278± 30 e | 52 ± 32 bc | 196 ± 90 f | 1.49 ± 0.07 b |
| | Rubel | 387 ± 47 c | 101 ± 21 a | 439 ± 156 c | 1.48 ± 0.16 ab |
| | Spartan | 330 ± 42 cd | 65 ± 35 bc | 274 ± 107 de | 1.48 ± 0.23 ab |
| | Sunshine Blue | 379± 39 c | 53 ± 18 bc | 389 ± 94 cd | 1.46 ± 0.29 ab |
| | Toro | 210± 31 f | 45 ± 25 bce | 180 ± 68 f | 1.43 ± 0.21 b |
| | Vernon | 437 ± 42 b | 82± 42 ab | 484 ± 147 bc | 1.34 ± 0.19 bc |
| YEARS | **2020** | 288 ± 115 b | 35 ± 16 b | 288 ± 190 ab | 1.40 ± 0.17 b |
| | **2021** | 319 ± 87 ab | 46 ± 21 b | 366 ± 238 a | 1.36 ± 0.20 b |
| | **2022** | 348 ± 99 a | 91 ± 30 a | 231 ± 155 b | 1.60 ± 0.16 a |

Legend: degrees of freedom (DF); values in bold indicate, for each trait, the source of variation with the largest mean square. * significant ($p < 0.05$); ** significant ($p < 0.01$); *** significant ($p < 0.001$); ns, not significant. Different letters within a column indicate significant differences ($p < 0.05$).

Marked variability was observed among the 35 genotypes for all bioactive compounds and for antioxidant activity (Table 3B). Total phenolic content (TPC) ranged from 191 mg GAE 100 $g^{-1}$ FW in Brigitta Blue to 544 mg GAE 100 $g^{-1}$ FW in Powder Blue, followed by Ochlockonee (533), Hortblue Petite (467) and Vernon (437 mg GAE 100 $g^{-1}$ FW), while the lowest values were recorded in Cosmopolitan, Toro and Liberty (204–212 mg GAE 100 $g^{-1}$ FW). Comparable ranges have been reported previously [9,36–39].

Total monomeric anthocyanins (TMA) ranged from only 7 mg C3GE 100 $g^{-1}$ FW in the pink-fruited Pink Lemonade to 101 mg C3GE 100 $g^{-1}$ FW in Rubel, with high values also in Early Blue (87), Duke and Vernon (82), and Hortblue Petite (80 mg C3GE 100 $g^{-1}$ FW). The

very low content of Pink Lemonade is consistent with its pink fruit color. TMA is known to depend on genotype, environment, and ripening stage [40]: a three-fold variation was reported among 13 commercial cultivars [41], and 90% of a collection of 80 highbush and 135 lowbush accessions differed by a factor of 1.6 [42].

Total flavan content (FLC) showed the widest range among the traits analyzed, from 92 mg CAE 100 $g^{-1}$ FW in Brigitta Blue to 797 mg CAE 100 $g^{-1}$ FW in Powder Blue, followed by Hortblue Petite (784), Ochlockonee (582), and Vernon (484 mg CAE 100 $g^{-1}$ FW). These values are broadly consistent with the flavonoid contents reported by Rossi et al. [9].

Associations between phenolic composition and antioxidant capacity have also been reported in other fruit species [43,44].

Antioxidant activity (AA) evaluated using the DPPH radical scavenging assay varied from 1.24 µg TE $mg^{-1}$ FW in Blue Ribbon to 1.69 µg TE $mg^{-1}$ FW in Nui, with high values also in Cipria (1.66) and Bluecrop (1.64 µg TE $mg^{-1}$ FW), and is comparable to values reported previously [9,36–39]. The genotypes with the highest TPC and FLC (Powder Blue, Ochlockonee, Hortblue Petite and Vernon) did not show the highest AA, which indicates that the DPPH response was not determined solely by the total phenolic and flavan pools. It should be noted that antioxidant activity was assessed exclusively through the DPPH assay, which measures the ability of compounds to scavenge a specific free radical under defined experimental conditions. Therefore, the results provide an estimate of antiradical activity rather than a comprehensive evaluation of the overall antioxidant potential of the fruit. The use of a single assay represents a limitation of the study, as different antioxidant mechanisms may be better captured by complementary methods such as ABTS, FRAP or ORAC. Consequently, the observed differences among genotypes should be interpreted specifically in terms of DPPH radical scavenging capacity.

Significant interannual variability was detected in the phytochemical profile. TPC increased steadily from 288 mg GAE 100 $g^{-1}$ FW in 2020 to 348 mg GAE 100 $g^{-1}$ FW in 2022, TMA more than doubled, from 35 to 91 mg C3GE 100 $g^{-1}$ FW, and AA reached its maximum in 2022 (1.60 µg TE $mg^{-1}$ FW), whereas FLC peaked in 2021 (366 mg CAE 100 $g^{-1}$ FW) and declined to 231 mg CAE 100 $g^{-1}$ FW in the following year. A comparable interannual pattern was reported for Asimina triloba grown at the same experimental site [19].

The accumulation of these compounds is influenced by irradiance and temperature, which regulate the synthesis of antioxidant metabolites [45,46]; flavonols, for instance, act as UV-B screening compounds and accumulate in response to the radiation intercepted by the developing fruit.

### *3.4. Chromatographic Evaluation of the Phenolic Profile*

The following section focuses exclusively on genotype-related differences in phytochemical composition, since the results indicate a pronounced cultivar effect on phenolic profile. Ochlockonee and Vernon showed the highest concentrations of hydroxycinnamic acids and of several anthocyanin derivatives, in agreement with Castrejón et al. [47]; Spartan and Powder Blue were also rich in anthocyanins, while Hortblue Poppins stood out for its flavonol content (Tables 4–6 and S2).

The mean hydroxycinnamic acid contents recorded over the three cropping years are reported in Table 4. Significant differences ($p \leq 0.05$) were observed among the genotypes investigated, highlighting considerable genotypic variability.

Hydroxycinnamic acids represented a major fraction of the phenolic profile in all cultivars, with chlorogenic acid as the predominant compound. Its concentration ranged from less than 1000 mg $kg^{-1}$ FW in Camellia and Hortblue Poppins to 3276.9 mg $kg^{-1}$ FW in Ochlockonee, followed by Vernon (3000.3), Bluecrop (2574.3), Powder Blue (2437.5) and Cooper (2322.2 mg $kg^{-1}$ FW). Neochlorogenic acid followed a different pattern, with

the highest value in Duke (73.1 mg kg$^{-1}$ FW) and several cultivars at very low or non-detectable levels, whereas cryptochlorogenic acid was most abundant in Ochlockonee (78.9 mg kg$^{-1}$ FW) and Vernon (55.5 mg kg$^{-1}$ FW).

**Table 4.** Hydroxycinnamic acid content (mg kg$^{-1}$ FW) of the 35 blueberry genotypes; values are means ± standard deviation over the three crop years; LSD, least significant difference ($p \leq 0.05$).

| Genotypes | Neochlorogenic Acid (mg kg$^{-1}$ FW) | Chlorogenic Acid (mg kg$^{-1}$ FW) | Cryptochlorogenic Acid (mg kg$^{-1}$ FW) | p-Coumaroylquinic Acid (mg kg$^{-1}$ FW) |
|---|---|---|---|---|
| **Aurora** | 20.0 ± 1.0 | 1942.1 ± 25.0 | 31.9 ± 9.4 | 15.8 ± 2.1 |
| **Berkeley** | 9.8 ± 0.6 | 1076.9 ± 472.9 | 23.5 ± 5.3 | 20.1 ± 2.5 |
| **Blue Ribbon** | nd | 1718.2 ± 381.0 | 27.8 ± 9.0 | 15.2 ± 1.9 |
| **Bluecrop** | nd | 2574.3 ± 234.8 | 45.1 ± 14.7 | 16.6 ± 1.1 |
| **Blueray** | nd | 948.3 ± 1030.5 | 33.2 ± 11.5 | 19.8 ± 2.2 |
| **Brigitta Blue** | nd | 1142.6 ± 164.2 | 21.3 ± 0.2 | 32.9 ± 3.6 |
| **Camellia** | 17.2 ± 0.9 | 844.5 ± 25.2 | 11.6 ± 0.2 | 20.3 ± 1.1 |
| **Chandler** | 16.4 ± 0.5 | 1300.0 ± 81.3 | 25.2 ± 4.1 | 14.6 ± 1.5 |
| **Cipria** | 53.8 ± 1.4 | 1856.6 ± 73.4 | 45.9 ± 9.7 | 21.7 ± 2.2 |
| **Cooper** | nd | 2322.2 ± 649.2 | 39.0 ± 4.6 | 14.9 ± 1.3 |
| **Cosmopolitan** | nd | 1002.6 ± 102.5 | 23.7 ± 0.2 | 11.4 ± 1.4 |
| **Coville** | nd | 1944.3 ± 280.1 | 25.8 ± 4.0 | 18.4 ± 3.8 |
| **Darrow** | nd | 1929.4 ± 360.1 | 32.0 ± 2.7 | 24.3 ± 1.5 |
| **Draper** | nd | 2274.9 ± 361.4 | 54.9 ± 19.7 | 15.5 ± 2.0 |
| **Duke** | 73.1 ± 1.8 | 1757.7 ± 144.0 | 50.0 ± 21.4 | 32.1 ± 22.4 |
| **Early Blue** | nd | 1408.8 ± 274.2 | 12.9 ± 10.2 | 16.6 ± 1.3 |
| **Elizabeth** | nd | 1868.1 ± 149.0 | 27.9 ± 2.0 | 17.7 ± 2.1 |
| **Elliot** | nd | 1562.6 ± 72.2 | 31.6 ± 3.8 | 16.6 ± 1.0 |
| **Hortblue Petite** | nd | 1270.4 ± 83.9 | 41.1 ± 6.4 | 15.6 ± 1.1 |
| **Hortblue Poppins** | 51.7 ± 1.6 | 800.8 ± 72.9 | 17.5 ± 8.8 | 30.7 ± 10.8 |
| **Legacy** | nd | 1766.0 ± 177.7 | 21.1 ± 1.2 | 16.1 ± 1.2 |
| **Liberty** | nd | 1113.9 ± 15.6 | 24.4 ± 9.2 | 17.1 ± 1.4 |
| **Nui** | nd | 1529.4 ± 596.2 | 36.7 ± 12.3 | 11.5 ± 0.9 |
| **Ochlockonee** | nd | 3276.9 ± 285.6 | 78.9 ± 3.6 | 13.2 ± 1.2 |
| **Ozark Blue** | nd | 1607.9 ± 98.9 | 33.3 ± 2.8 | 14.3 ± 1.5 |
| **Patriot** | nd | 1609.4 ± 274.1 | 40.9 ± 8.1 | 15.3 ± 1.5 |
| **Pink Lemonade** | 24.9 ± 1.2 | 1262.5 ± 481.3 | 36.4 ± 9.7 | 17.6 ± 7.3 |
| **Powder Blue** | nd | 2437.5 ± 368.0 | 54.6 ± 18.1 | 16.4 ± 2.4 |
| **Rebel** | 29.0 ± 1.1 | 1273.4 ± 168.2 | 17.0 ± 4.5 | 25.2 ± 7.4 |
| **Roxy Blue** | nd | 2028.9 ± 162.7 | 37.6 ± 1.3 | 14.3 ± 0.6 |
| **Rubel** | nd | 1855.9 ± 77.2 | 40.0 ± 15.8 | 17.9 ± 1.2 |
| **Spartan** | nd | 1430.3 ± 175.4 | 24.5 ± 5.3 | 17.8 ± 1.5 |
| **Sunshine Blue** | nd | 2256.9 ± 308.9 | 43.7 ± 6.4 | 20.8 ± 1.1 |
| **Toro** | 11.2 ± 0.5 | 1038.2 ± 37.7 | 27.4 ± 1.3 | 15.4 ± 1.3 |
| **Vernon** | 30.1 ± 1.3 | 3000.3 ± 17.5 | 55.5 ± 14.0 | 21.3 ± 1.2 |
| **LSD ($p \leq 0.05$)** | 1.9 | 510.6 | 15.7 | 11.2 |

Among the minor hydroxycinnamic acid derivatives, p-coumaroylquinic acid showed significant genotypic variability, with the highest concentrations recorded in Brigitta Blue (32.9 mg kg$^{-1}$ FW), Duke (32.1 mg kg$^{-1}$ FW), and Hortblue Poppins (30.7 mg kg$^{-1}$ FW), followed by Rebel (25.2 mg kg$^{-1}$ FW) and Darrow (24.3 mg kg$^{-1}$ FW).

The present findings are partially consistent with those reported by You et al. [48] and Grace et al. [49]; the quantitative differences observed probably reflect differences in sample preparation, extraction, chromatographic separation and detection, as well as in genetic background and growing conditions.

**Table 5.** Flavan-3-ol and procyanidin content (mg kg$^{-1}$ FW) of the 35 blueberry genotypes; values are means ± standard deviation over the three crop years; LSD, least significant difference ($p \leq 0.05$).

| Genotypes | (+)-Catechin (mg kg$^{-1}$ FW) | (−)-Epicatechin (mg kg$^{-1}$ FW) | Procyanidin B1 (mg kg$^{-1}$ FW) | Procyanidin B2 (mg kg$^{-1}$ FW) |
|---|---|---|---|---|
| **Aurora** | 14.5 ± 0.7 | 6.4 ± 1.1 | 22.1 ± 2.4 | 15.2 ± 9.5 |
| **Berkeley** | 58.6 ± 26.5 | 12.5 ± 3.2 | 59.3 ± 32.3 | 19.6 ± 3.9 |
| **Blue Ribbon** | 20.0 ± 7.5 | 4.4 ± 1.7 | 25.7 ± 1.4 | 8.5 ± 2.9 |
| **Bluecrop** | 96.4 ± 15.3 | 23.8 ± 0.7 | 94.4 ± 17.8 | 38.8 ± 2.0 |
| **Blueray** | 42.3 ± 8.6 | 10.9 ± 0.4 | 51.0 ± 17.1 | 19.9 ± 0.3 |
| **Brigitta Blue** | 9.4 ± 2.1 | 4.5 ± 3.0 | 11.6 ± 0.1 | 11.6 ± 9.6 |
| **Camellia** | 43.7 ± 4.7 | 29.9 ± 5.4 | 50.9 ± 10.0 | 51.0 ± 0.7 |
| **Chandler** | 74.7 ± 10.1 | 10.2 ± 2.0 | 86.5 ± 30.0 | 16.0 ± 2.1 |
| **Cipria** | 45.4 ± 14.0 | 23.3 ± 10.5 | 51.9 ± 24.4 | 34.6 ± 13.3 |
| **Cooper** | 65.8 ± 8.4 | 38.6 ± 10.4 | 67.5 ± 21.9 | 53.4 ± 16.4 |
| **Cosmopolitan** | 17.9 ± 3.7 | 3.6 ± 0.1 | 19.8 ± 0.7 | 9.3 ± 3.5 |
| **Coville** | 88.2 ± 30.2 | 5.9 ± 6.2 | 74.1 ± 2.0 | 19.2 ± 1.9 |
| **Darrow** | 66.5 ± 6.0 | 13.9 ± 0.1 | 73.6 ± 1.3 | 17.9 ± 1.8 |
| **Draper** | 18.3 ± 5.8 | 10.8 ± 2.1 | 15.8 ± 1.3 | 12.6 ± 3.7 |
| **Duke** | 37.8 ± 7.8 | 8.8 ± 2.9 | 52.3 ± 7.0 | 16.9 ± 4.6 |
| **Early Blue** | 14.6 ± 1.5 | 4.1 ± 1.3 | 15.6 ± 1.7 | 9.6 ± 8.0 |
| **Elizabeth** | 21.4 ± 3.6 | 7.1 ± 1.5 | 24.0 ± 1.4 | 10.3 ± 1.2 |
| **Elliot** | 18.4 ± 4.3 | 6.2 ± 0.9 | 23.7 ± 4.6 | 10.9 ± 4.5 |
| **Hortblue Petite** | 135.1 ± 13.1 | 40.4 ± 14.7 | 97.0 ± 117.6 | 65.2 ± 32.2 |
| **Hortblue Poppins** | 12.0 ± 2.7 | 10.0 ± 1.8 | 13.2 ± 1.7 | 13.2 ± 1.5 |
| **Legacy** | 34.1 ± 4.9 | 11.8 ± 1.9 | 40.7 ± 1.4 | 21.2 ± 0.6 |
| **Liberty** | 17.5 ± 2.3 | 8.7 ± 1.2 | 17.4 ± 5.0 | 15.3 ± 4.3 |
| **Nui** | 77.8 ± 12.3 | 16.7 ± 0.5 | 77.1 ± 6.5 | 24.0 ± 2.1 |
| **Ochlockonee** | 20.5 ± 5.9 | 19.9 ± 4.3 | 17.1 ± 1.2 | 26.5 ± 3.8 |
| **Ozark Blue** | 62.9 ± 6.5 | 12.9 ± 0.2 | 68.0 ± 16.4 | 18.1 ± 1.3 |
| **Patriot** | 60.4 ± 12.6 | 26.0 ± 1.8 | 86.8 ± 12.2 | 44.7 ± 4.3 |
| **Pink Lemonade** | 29.9 ± 11.2 | 7.8 ± 4.4 | 37.5 ± 18.3 | 15.0 ± 8.6 |
| **Powder Blue** | 83.8 ± 5.7 | 29.0 ± 2.5 | 81.3 ± 11.0 | 24.4 ± 0.4 |
| **Rebel** | 45.8 ± 10.0 | 28.1 ± 6.3 | 54.3 ± 20.5 | 55.7 ± 10.8 |
| **Roxy Blue** | 16.7 ± 2.4 | 7.8 ± 1.8 | 16.6 ± 7.3 | 11.0 ± 3.4 |
| **Rubel** | 40.8 ± 47.6 | 19.3 ± 0.7 | 77.0 ± 14.7 | 15.0 ± 17.2 |
| **Spartan** | 28.4 ± 4.1 | 7.4 ± 1.5 | 41.3 ± 6.4 | 12.1 ± 1.1 |
| **Sunshine Blue** | 17.6 ± 1.5 | 16.4 ± 3.1 | 22.7 ± 5.2 | 25.2 ± 1.5 |
| **Toro** | 66.2 ± 9.0 | 8.0 ± 0.6 | 79.1 ± 23.4 | 14.9 ± 0.4 |
| **Vernon** | 82.8 ± 11.7 | 20.9 ± 2.4 | 69.5 ± 8.1 | 19.2 ± 4.1 |
| **LSD ($p \leq 0.05$)** | 21.1 | 8.1 | 38.8 | 14.4 |

Table 5 summarizes the concentrations of flavan-3-ols and procyanidins detected in the investigated blueberry genotypes. Flavan-3-ols, together with their oligomeric and polymeric derivatives known as procyanidins, are among the most relevant flavonoid compounds found in berry species and are recognized for their considerable biological and antioxidant properties [50–52].

(+)-Catechin was the predominant flavan-3-ol, ranging from 9.4 mg kg$^{-1}$ FW in Brigitta Blue to 135.1 mg kg$^{-1}$ FW in Hortblue Petite, with high values also in Bluecrop (96.4), Coville (88.2), Powder Blue (83.8) and Vernon (82.8 mg kg$^{-1}$ FW). (−)-Epicatechin followed a similar pattern at lower concentrations, from 3.6 mg kg$^{-1}$ FW in Cosmopolitan to 40.4 mg kg$^{-1}$ FW in Hortblue Petite.

Among the procyanidins, procyanidin B1 was generally the most abundant dimer, ranging from 11.6 mg kg$^{-1}$ FW in Brigitta Blue to 97.0 mg kg$^{-1}$ FW in Hortblue Petite, with values above 77 mg kg$^{-1}$ FW also in Bluecrop, Patriot, Chandler, Powder Blue, Toro, Nui and Rubel.

**Table 6.** Flavonol content (mg kg$^{-1}$ FW) of the 35 blueberry genotypes; values are means ± standard deviation over the three crop years; LSD, least significant difference ($p \leq 0.05$).

| Genotypes | Quercetin-3-O-Rutinoside | Quercetin-3-O-Glucoside | Kaempferol Rutinoside |
|---|---|---|---|
| **Aurora** | 40.5 ± 12.7 | 35.8 ± 10.9 | 6.9 ± 2.5 |
| **Berkeley** | 4.3 ± 2.5 | 10.7 ± 4.4 | 0.4 ± 0.04 |
| **Blue Ribbon** | 13.5 ± 13.2 | 32.1 ± 9.0 | 2.3 ± 0.4 |
| **Bluecrop** | 31.1 ± 5.4 | 23.6 ± 28.7 | 2.3 ± 0.3 |
| **Blueray** | 14.8 ± 5.4 | 16.7 ± 4.9 | 1.3 ± 0.6 |
| **Brigitta Blue** | 44.9 ± 23.4 | 36.9 ± 23.0 | 7.7 ± 4.7 |
| **Camellia** | 21.0 ± 0.6 | 25.0 ± 0.4 | 2.5 ± 0.4 |
| **Chandler** | 8.3 ± 1.1 | 10.0 ± 11.9 | 0.9 ± 0.4 |
| **Cipria** | 28.4 ± 6.1 | 27.1 ± 7.0 | 5.7 ± 1.2 |
| **Cooper** | 16.0 ± 0.3 | 23.2 ± 1.8 | 1.6 ± 0.2 |
| **Cosmopolitan** | 80.9 ± 2.4 | 19.2 ± 0.8 | 4.0 ± 0.1 |
| **Coville** | 21.7 ± 6.4 | 35.8 ± 0.3 | 1.8 ± 0.6 |
| **Darrow** | 38.7 ± 1.4 | 32.4 ± 1.4 | 4.7 ± 0.0 |
| **Draper** | 15.8 ± 0.5 | 14.3 ± 0.6 | 3.1 ± 1.6 |
| **Duke** | 7.4 ± 2.8 | nd | 0.9 ± 0.03 |
| **Early Blue** | 15.8 ± 0.6 | 32.4 ± 6.9 | 1.2 ± 0.1 |
| **Elizabeth** | 8.2 ± 0.1 | 11.0 ± 2.0 | 1.5 ± 0.3 |
| **Elliot** | 16.2 ± 3.4 | 17.0 ± 2.5 | 1.4 ± 0.3 |
| **Hortblue Petite** | 7.4 ± 3.4 | 18.8 ± 9.4 | 0.8 ± 0.4 |
| **Hortblue Poppins** | 89.4 ± 4.6 | 62.7 ± 3.1 | 18.3 ± 0.5 |
| **Legacy** | 12.9 ± 2.0 | 26.9 ± 4.9 | 2.5 ± 0.4 |
| **Liberty** | 48.4 ± 9.9 | 32.4 ± 4.4 | 4.2 ± 1.0 |
| **Nui** | 49.4 ± 0.5 | 16.2 ± 0.6 | 2.1 ± 0.1 |
| **Ochlockonee** | 30.4 ± 2.6 | 32.9 ± 2.1 | 4.0 ± 0.6 |
| **Ozark Blue** | 12.3 ± 2.8 | 92.8 ± 97.8 | 1.8 ± 0.4 |
| **Patriot** | 30.3 ± 1.1 | 17.6 ± 0.6 | 2.2 ± 0.1 |
| **Pink Lemonade** | 6.2 ± 1.1 | 4.4 ± 0.2 | 1.1 ± 0.2 |
| **Powder Blue** | nd | 2.7 ± 0.1 | nd |
| **Rebel** | 25.4 ± 1.0 | 30.9 ± 0.7 | 2.9 ± 0.3 |
| **Roxy Blue** | 23.9 ± 10.4 | 46.2 ± 15.3 | 1.6 ± 0.9 |
| **Rubel** | 21.8 ± 1.2 | 34.9 ± 0.6 | 2.7 ± 0.3 |
| **Spartan** | 8.0 ± 7.9 | 7.1 ± 1.2 | 0.2 ± 0.04 |
| **Sunshine Blue** | 1.5 ± 0.7 | 9.4 ± 0.4 | nd |
| **Toro** | 2.3 ± 0.5 | 5.6 ± 1.9 | nd |
| **Vernon** | 4.2 ± 2.4 | 4.5 ± 0.3 | 0.5 ± 0.03 |
| **LSD ($p \leq 0.05$)** | 10.2 | 10.4 | 0.8 |

Procyanidin B2 ranged from 8.5 mg kg$^{-1}$ FW in Blue Ribbon to 65.2 mg kg$^{-1}$ FW in Hortblue Petite, with high values also in Rebel (55.7), Cooper (53.4) and Camellia (51.0 mg kg$^{-1}$ FW); most of the remaining genotypes contained less than 30 mg kg$^{-1}$ FW.

Bluecrop, Powder Blue, Patriot, Nui, Vernon and Hortblue Petite consistently showed the highest concentrations of flavan-3-ols and procyanidins, which indicates a strong genotypic control of their accumulation and identifies these cultivars as the richest sources within the collection.

The present findings corroborate earlier studies conducted on strawberry and blueberry fruits, which similarly identified flavan-3-ols and procyanidins among the major phenolic constituents of these berry species [51–54].

It should be noted that only the monomeric flavan-3-ols and the B1 and B2 dimers were quantified, whereas in blueberry fruit the proanthocyanidin fraction also comprises oligomers and polymers with a higher degree of polymerization [55]. The values reported here therefore describe the low-molecular-weight fraction of the flavan-3-ol pool and underestimate the total proanthocyanidin content, which also accounts for part of the difference

with respect to the FLC values obtained by the vanillin assay, since the latter responds to a broader range of flavan structures.

The concentrations of the main flavonols identified in the investigated blueberry genotypes, namely quercetin 3-O-rutinoside, quercetin 3-O-glucoside, and kaempferol rutinoside, are reported in Table 6. Significant differences among genotypes were observed for all compounds, as indicated by the respective LSD values ($p \leq 0.05$), highlighting a strong genotypic influence on flavonol accumulation.

Quercetin 3-O-rutinoside ranged from 1.5 mg $kg^{-1}$ FW in Sunshine Blue to 89.4 mg $kg^{-1}$ FW in Hortblue Poppins, with high values also in Cosmopolitan (80.9), Nui (49.4), Liberty (48.4) and Brigitta Blue (44.9 mg $kg^{-1}$ FW), and was not detected in Powder Blue. Quercetin 3-O-glucoside occurred at concentrations comparable to or higher than those of the rutinoside, reaching 92.8 mg $kg^{-1}$ FW in Ozark Blue and 62.7 mg $kg^{-1}$ FW in Hortblue Poppins, while it was not detected in Duke. Kaempferol rutinoside was consistently the least abundant, with a maximum of 18.3 mg $kg^{-1}$ FW in Hortblue Poppins and no detectable amounts in Powder Blue, Sunshine Blue and Toro.

Hortblue Poppins showed the highest content of all three flavonols, followed by Aurora, Brigitta Blue, Liberty, Cosmopolitan and Ozark Blue, which confirms the role of the genetic background in flavonol accumulation.

The flavonol concentrations observed in the present study are generally consistent with previous reports on blueberry germplasm, where quercetin glycosides were identified as the predominant flavonol compounds and substantial cultivar-dependent variability was reported [52,56,57]. Differences among studies may be attributed to genotype, growing conditions, fruit maturity, and analytical methodologies employed for flavonol extraction and quantification.

The anthocyanin content of the individual genotypes is reported in Table S2 of the Supplementary Materials, and the results are summarized below by anthocyanidin class.

Summed over the glycosides of each anthocyanidin, petunidin derivatives were the most abundant class (mean 1391 mg $kg^{-1}$ FW across genotypes), followed by malvidin (1121 mg $kg^{-1}$ FW), cyanidin (808 mg $kg^{-1}$ FW), delphinidin (727 mg $kg^{-1}$ FW), peonidin (648 mg $kg^{-1}$ FW) and pelargonidin derivatives (269 mg $kg^{-1}$ FW). Spartan showed the highest totals for the petunidin, malvidin, delphinidin and peonidin classes (1799, 1574, 955 and 828 mg $kg^{-1}$ FW, respectively), whereas Powder Blue ranked first for the cyanidin and pelargonidin classes (1454 and 307 mg $kg^{-1}$ FW). The lowest totals were recorded in Pink Lemonade for the delphinidin, cyanidin, petunidin, and peonidin classes, consistent with its pink phenotype, and in Darrow for the malvidin class.

Significant differences among genotypes were detected for all identified anthocyanins (LSD, $p \leq 0.05$), confirming the strong influence of genetic background on anthocyanin biosynthesis and accumulation. Overall, the anthocyanin composition was dominated by glycosylated derivatives of cyanidin, delphinidin, petunidin, peonidin, and malvidin, which are known to represent the major anthocyanin classes in highbush blueberries [55]. A pelargonidin hexoside was also detected and quantified; this anthocyanidin is uncommon in Vaccinium, and its identification was supported by the aglycone fragment at m/z 271 in the MS/MS spectrum.

Cyanidin-3-O-glucoside was among the most abundant anthocyanins and varied markedly among cultivars [14,58,59], with the highest concentrations in Powder Blue (883.8 mg $kg^{-1}$ FW), Ochlockonee (869.6), Vernon (758.1), Draper (689.4) and Hortblue Petite (686.1 mg $kg^{-1}$ FW), and the lowest in Pink Lemonade, Cosmopolitan, Chandler and Darrow.

Considerable variation was also detected for cyanidin-3-O-pentoside, ranging from 219.8 mg $kg^{-1}$ FW in Pink Lemonade to 570.0 mg $kg^{-1}$ FW in Powder Blue. Elevated

levels were additionally found in Hortblue Petite (564.6 mg $kg^{-1}$ FW), Ochlockonee (523.8 mg $kg^{-1}$ FW), and Vernon (491.9 mg $kg^{-1}$ FW).

Among the delphinidin derivatives, delphinidin-3-O-pentoside reached 537.9 mg $kg^{-1}$ FW in Spartan, with values above 450 mg $kg^{-1}$ FW also in Cipria, Cooper, Blueray, Berkeley, Hortblue Petite and Hortblue Poppins, while delphinidin-3-O-hexoside was highest in Nui (492.0 mg $kg^{-1}$ FW). Petunidin-3-O-hexoside 1 was one of the predominant compounds, reaching 780.4 mg $kg^{-1}$ FW in Spartan. Malvidin derivatives were quantitatively dominant: malvidin-3-O-hexoside 1 reached its maximum in Spartan (962.0 mg $kg^{-1}$ FW), followed by Early Blue (735.9) and Powder Blue (702.8 mg $kg^{-1}$ FW), whereas malvidin-3-O-arabinoside was highest in Ochlockonee (411.7 mg $kg^{-1}$ FW) and Vernon (394.4 mg $kg^{-1}$ FW).

Peonidin-3-O-hexoside reached its highest concentrations in Spartan (538.5 mg $kg^{-1}$ FW), Hortblue Petite (448.5), Camellia (466.9) and Early Blue (447.9 mg $kg^{-1}$ FW), and its lowest values in Pink Lemonade, Darrow and Roxy Blue.

Overall, Spartan, Powder Blue, Hortblue Petite, Vernon, Ochlockonee and Draper showed the highest concentrations across several anthocyanin classes, whereas Pink Lemonade, Chandler, Cosmopolitan and Darrow showed the lowest, in agreement with previous reports of a strong genotypic influence on anthocyanin composition [14,58,59].

The predominance of malvidin and delphinidin derivatives, and the position of the rabbiteye genotypes among those with the highest anthocyanin concentrations, are consistent with the differentiation between rabbiteye and highbush anthocyanin profiles described by Pertuzatti et al. [60], who separated the two groups mainly on the basis of the relative abundance of the individual glycosides rather than on total anthocyanin content. The low total anthocyanin content of Pink Lemonade is consistent with its pink phenotype; this cultivar nevertheless retained appreciable concentrations of hydroxycinnamic acids and flavonols, which indicates that the trait involves the anthocyanin branch of the flavonoid pathway rather than a general reduction in phenolic metabolism.

Pearson's correlation analysis revealed that the antioxidant activity (AA) was only weakly associated with the concentrations of the individual phenolic compounds identified by LC-MS. In general, most correlation coefficients were low ($|r| < 0.30$), suggesting that the antioxidant capacity of blueberry fruits cannot be explained by a single phenolic constituent but rather reflects the combined contribution of multiple compounds and their interactions. Among the analyzed metabolites, delphinidin-3-O-pentoside showed the strongest negative correlation with AA ($r = -0.271$, $p = 0.0196$), followed by petunidin-3-O-arabinoside ($r = -0.266$, $p = 0.0249$) and petunidin-3-O-hexoside 1 ($r = -0.206$, $p = 0.0818$). Although statistically significant in some cases, these relationships were relatively weak, indicating a limited contribution of individual anthocyanins to the overall antioxidant response. Negative but non-significant correlations were also observed for several other anthocyanins, including cyanidin-3-O-glucoside ($r = -0.173$), petunidin-3-O-rhamnoside ($r = -0.172$), cyanidin-3-O-pentoside ($r = -0.143$), malvidin-3-O-arabinoside ($r = -0.138$), and peonidin-3-O-arabinoside ($r = -0.123$). Similarly, hydroxycinnamic acids such as neochlorogenic acid ($r = -0.074$), chlorogenic acid ($r = -0.118$), cryptochlorogenic acid ($r = -0.076$), and *p*-coumaroylquinic acid ($r = -0.165$) exhibited weak associations with antioxidant activity. Among flavan-3-ols, (+)-catechin ($r = -0.070$) and procyanidin B1 ($r = -0.033$) were poorly related to AA, whereas (−)-epicatechin showed a small positive correlation ($r = 0.071$). Likewise, procyanidin B2 displayed a weak positive association ($r = 0.093$). Flavonols were not significantly correlated with antioxidant activity, with correlation coefficients ranging from −0.160 for kaempferol rutinoside to 0.015 for quercetin-3-O-rutinoside. Overall, these findings indicate that antioxidant activity was not strongly determined by the concentration of any single phenolic compound. The absence of strong correlations suggests that the antioxidant response is likely influenced by the synergistic effects among different phenolic

classes, their structural characteristics, and the relative abundance of the various metabolites present in the fruit. This observation explains why cultivars characterized by high total phenolic, anthocyanin, or flavan contents did not necessarily exhibit the highest antioxidant activity values.

### 3.5. Relationships Between Fruit Composition and Climatic Variables

The relationships between the meteorological conditions of the ripening period and the quality-related traits were examined by comparing the annual means of the traits (Tables 2B and 3B) with the corresponding seasonal values of the climatic variables (Figure 1). Because the three crop years provide only three distinct climatic profiles, these relationships are described qualitatively and no model was fitted.

The 2022 season, which recorded the highest mean and maximum temperatures and the highest solar radiation during the ripening period, together with the lowest rainfall and relative humidity, showed the highest total phenolic content, the highest total monomeric anthocyanin content and the highest antioxidant activity, as well as the largest berries and the lowest soluble solids content and titratable acidity. The 2020 season, cooler and wetter in the same phase, showed the opposite pattern, and 2021 was intermediate for most traits. Consistent with the analysis of variance, crop year was a major source of variation for TPC, TMA and AA: across the three seasons, temperature and solar radiation of the ripening period varied in the same direction as these traits, whereas rainfall and relative humidity varied in the opposite direction.

It should be noted that these results were obtained over only three growing seasons and therefore provide a preliminary assessment of the relationships between meteorological conditions and cultivar performance. A more comprehensive evaluation of climatic effects on blueberry cultivars would require a longer monitoring period encompassing a wider range of environmental conditions and year-to-year variability.

The association between the 2022 season and the highest total phenolic and anthocyanin content deserves further comment. High temperature during ripening is generally reported to limit anthocyanin accumulation in fruit, whereas irradiance stimulates flavonoid biosynthesis [61,62]. In the present trial, the two factors varied concurrently, 2022 being both the warmest and the most irradiated season, and the net effect on anthocyanin accumulation was positive. This suggests that, under the thermal regime of central Italy, the stimulation exerted by solar radiation prevailed over the inhibition attributed to temperature. The two effects cannot be separated in an observational design, and controlled environment or shading experiments would be required to quantify their relative contributions.

## 4. Conclusions

The three-year evaluation of 35 blueberry genotypes showed that both the genetic background and the environmental conditions influence fruit quality, phytochemical composition and antioxidant potential. Genotype was the predominant source of variation for most pomological traits, while the chemical profile was mainly influenced by the growing season. Conversely, flavan content was likewise dominated by the crop year, while the genotype × year interaction, although significant for all traits, accounted for smaller mean squares, indicating that the ranking of the genotypes was only partly conserved across seasons. Warmer, more irradiated and drier conditions were associated with larger berries, lower soluble solids and acidity, and with the highest phenolic, anthocyanin and antioxidant values. Powder Blue, Ochlockonee, Vernon, Hortblue Petite, Rubel and Nui exhibited the most favorable phytochemical profiles, whereas Pink Lemonade displayed a distinctive composition related to its pigmentation. Across the three seasons, this variation followed the same direction as the temperature and solar radiation of the ripening period.

The scope of these results is defined by the experimental design. The phenolic profile was obtained by targeted quantification against authentic standards and therefore covers a defined subset of compounds. The genotype × environment interaction was estimated over three consecutive seasons at a single site and thus describes the response of the cultivars to interannual climatic variation; its extension to other growing areas requires evaluation across multiple sites.

Overall, the findings emphasize the importance of integrating genotype × environment interactions into blueberry breeding and cultivar selection. Genotypes combining high fruit quality, phytochemical richness, antioxidant capacity, and stable performance across contrasting climatic conditions represent valuable resources for developing cultivars adapted to increasingly variable production environments. It should be noted that the present results were obtained under the specific environmental and agronomic conditions of central Italy. Therefore, the performance of the evaluated cultivars may vary in other growing environments, and further research under different pedoclimatic conditions is needed to confirm the observed trends.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app162211412/s1. Table S1: Phenolic compounds identified and quantified in blueberry fruit by LC–QTOF-MS (compound class, molecular formula, measured m/z, ionization mode and retention time); Table S2: Anthocyanin content (mg $kg^{-1}$ FW) of the 35 blueberry genotypes; values are means ± standard deviation over the three crop years (2020–2022). Different letters within a column indicate significant differences among genotypes (LSD, $p \leq 0.05$).

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