YIELD AND NUTRITIONAL QUALITY OF HIGHBUSH BLUEBERRY GENOTYPES TRIALLED IN MEDITERRANEAN HOT SUMMER CLIMATE

Influence of genotype on Blueberries performance in a Mid-Adriatic area in Italy

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ABSTRACT

Background: Cultivation of highbush blueberry (*Vaccinium corymbosum* L.) is increasing in Europe in the last years, in particular due to the availability of new genotypes suitable for the cultivation in many different environmental conditions. The aim of this study was to evaluate the resilience and nutritional quality of eleven highbush blueberry cultivars and two new selections (from The New Zealand Institute for Plant & Food Research Ltd breeding program) to Mediterranean hot summer climate conditions, by measuring: plant yield; seasonality; fruit sensorial traits; phytochemical content in fruits.

Results: The new blueberry genotype PFR005 showed high adaptability to these environmental conditions, with the highest total plant yield, while PFR075 was the best genotypes for the nutritional characteristics. Among cultivars, 'Cosmopolitan' showed the maximum average fruit weight, 'Blueray' and 'Hortblue Poppins' demonstrated a good sensorial profile, while the best cultivars from the nutritional point of view were 'Hortblue Poppins', 'Hortblue Petite' and 'Early Blue'.

Conclusion: Cultivated varieties or new genotypes could be suitable to satisfy the needs of different actors of the productive chain. The integration of the germplasm evaluation with tailored breeding program will help in the next future to create new cultivars useful to expand blueberry cultivation in Mediterranean hot summer climate conditions, up to now of high limitation for this crop.

Keywords: *Vaccinium*; plant yield; fruit quality; phytochemicals; phenolic acids; vitamin C

INTRODUCTION

Cultivated highbush blueberries (*Vaccinium corymbosum)* are produced commercially in Europe and recently the total blueberries production is duplicated, increasing from 47970 tons in 2010 to 100304 tons in 2017 (http://www.fao.org/faostat/en/#data/QC) (Figure 1). This growth trend has continued in recent years and has been accentuated by the diffusion of new genotypes that adapt to Mediterranean hot summer climate. The growth of blueberry cultivation in lower chill areas around the world has contributed to the release of cultivars that are suitable for those environments [1]. However, newly released low chill blueberry cultivars might not be available in specific territories and the increased demand for blueberry cultivation areas is limited by the low adaptability of the common knowledge varieties to different soils and climates. The grower's access to those varieties is in fact crucial for the success of the commercial establishment of a blueberry farm. A blueberry plantation of resilient cultivars has the potential to produce a good crop for many years and thus planting the most suitable genotypes is fundamental. For the growers, the most important traits of a cultivar are related to the berry plant yield efficiency, as the high plant yield, a high harvest speed, good average fruit weight and resistance to pest and diseases [2].

Blueberry fruits are believed to be good for human health for their high content of polyphenolic compounds. Amongst the polyphenolic compounds, anthocyanins provide blueberries with their characteristic blue color and have been shown to contribute to the antioxidant capacity of berry fruit [3]. In general, the health value of anthocyanins has been reviewed [4]. Those compounds are reported to have a role in improving circulation [5], preventing stroke [6], providing benefits to vision [5], and their anti-inflammatory and anti-oxidative effects are extensively reported [7,8].

Researches show that there is an interest in the anthocyanin content of blueberry fruit and that changes are expected among fruits of different cultivars, harvested in different seasons and cultivated under different conditions [9, 10]. Blueberry fruit is also rich in phenolic acids, a group of phenolic compounds that possesses free radical scavenging activity promoting human health benefits. Chlorogenic acid, the most prominent phenolic acid found in blueberry [11], resulted to slow the release of glucose into the bloodstream after meals [12]. The content of vitamin C is another parameter considered for the assessment of the nutritional quality of blueberries, even if there is a significant variability among *Vaccinium* species and cultivars [13]. In particular, the vitamin C content seems to be influenced mainly by the high brightness and the high pre-harvest temperature [14], and by cracking of the blueberry skin [13]. The increase of consumer attention to the healthy

aspects of fruit, and the demand of new fruit with high concentrations of health-promoting phytochemicals, are reasons inspiring breeders to develop new tailored breeding programs [15].

Only in the last recent years, the highbush blueberry cultivation has been extended to central- Italy and integrated with other berry fruit crops (i.e. strawberry, raspberry and blackberry). This climatic area is considered of high potential in differentiating blueberry harvesting period, to anticipate the release on the market of high-quality fruits. However, pedological characteristics frequently remains the main limiting factors for expanding blueberry cultivation. New breeding program for this crop must be finalized to the selection of new genotypes with increased adaptability to warmer climatic conditions and less acid – chalky soils, maintaining high yield and fruit nutritional quality.

On the light of those considerations, an experimental trial was set for testing thirteen highbush genotypes, combining some well-known commercial cultivars, more productive, and some newly released cultivars and advanced selections which are known to be suitable for mid chilling conditions, with fruits of good flavor and high phytochemical content [9].

The aim of this study is to report the results of the evaluation of highbush blueberry and to find genotypes suited to grow in hot summer Mediterranean climate and not acid soils. The genotypes performances were compared by evaluating: (1) the plant yield; (2) the seasonality; (3) the fruit qualitative traits; (4) the phytochemical content in fruits.

MATERIALS AND METHODS

Plant material

Cultivars and selections sourced from different nurseries were planted in 2010 at the "P. Rosati" Experimental Farm in Agugliano, Italy (43°31'N 13°36'E. 46 m altitude). According to the Köppen climate classification, this area falls within the climatic group "Csa" (Mediterranean hot summer climate). The soil characteristics at the "P. Rosati" Experimental Farm are typical of the Italian mid-Adriatic area: pH 7.9, active calcium 9%, texture composed by 40% clay, 25% sand and 35% silt. Those soil characteristics are not suitable for growing highbush blueberry. For this reason, plants were planted in trenches filled with a mixture of peat (65%), pumice (35%) and leonardite (5 kg m^{-3}). Trenches were 30 cm wide, 35 cm deep and spaced at 3.5 m between the rows. Plant density was 2.857 plants ha^{-1}. Every month, a Sulphuric ammendant (Sulfer 90®, Intertec International - Italy) has been distributed (40 kg ha^{-1}).

A drip irrigation and fertigation system of 120 kg ha^{-1} nitrogen, 120 kg ha^{-1} P_2O_5, 180 kg ha^{-1} K_2O was used as the basic agronomic technique.

In this study eleven northern highbush varieties were evaluated: 'Duke', 'Hortblue Petite', and 'Nui' were early precocity genotypes; 'Blueray', 'Cosmopolitan', 'Early Blue', 'Patriot', and 'Roxy Blue' were mid precocity genotypes; 'Blue Silk', 'Bluecrop', and 'Hortblue Poppins' were late precocity genotypes. 'Hortblue Poppins', 'Roxy Blue', 'Hortblue Petite', 'Blue Silk', and 'Cosmopolitan' were released by the New Zealand Plant and Food breeding program. In addition to those eleven cultivars, two new selections always from Plant and Food breeding program were planted in the experimental block (PFR005 and PFR075). Plants were planted in a complete randomized block design, with two plots of five plants each, for all genotypes.

Fruit harvest

The plant production was monitored for two seasons (2014 and 2015) characterized by different climatic conditions. At each year, the environmental conditions were recorded at site. Air temperature was measured hourly in 2 m height directly in the field, through the installation of a Data Logger "testo 175T1" (Lenzkirch, Germany). Temperature data were used to calculate growing degree days (GDD) based on 3°C as used by Gough [16]. GDDs were calculated from the first January of each year until harvest starts, in both 2014 and 2015 years. Another important parameter linked to the fruit harvest is the Precocity Index (PI); PI represent the average of the weighted days number needed to collect the whole production of a cultivar, from January 1, according to the following equation:

$$PI = \Sigma (Zqx)/Q$$

Where Z = number of elapsed days since January 1, q = total harvests production at the date Z, Q = total Production of all harvests.

Within each season, multiple pickings were necessary to complete the total fruit harvest for each genotype. Fully mature fruit were harvested in June and July, the total plant yield recorded (g fruits/plant), and the Average Fruit Weight (AFW, grams) assessed at each harvest. In order to identify the different ripening time of all genotype, the first harvest date is reported for each year and as mean of the two years (Tables 1 and 2).

For each plot, a subsample consisting of 300 g of undamaged fruits from the first, second and third main pickings were bulked and frozen at -20 °C for the phytochemical analyses, while the remaining undamaged fruits were frozen at -20°C for the quality parameters evaluation.

Fruit Quality Parameters

The fruit quality parameters were studied on undamaged fruit samples, harvested at ripening stage, including pooled fruit of the three main harvests.

For the soluble solid content (SSC), the juice of the defrosted blueberries was squeezed out. One or two drops of juice were put on the surface of a hand-held refractometer (model N-1 E, Atago Co., Tokyo, JP, automatic temperature compensation). The quantity of SSC is expressed in °Brix (%).

The titratable acidity (TA) was determined with the automatic titrator HI 84532 Fruit Juice Titratable Acidity (Hanna Instruments, Woonsocket, Rhode Island, USA). Briefly, 5 mL of the juice obtained above was put in a plastic Becker, and 45 mL of ultrapure water were added. This solution was titrated automatically by the instrument through the titrating solution provided by the manufacturer, until pH 8. The acidity is expressed as percentage of Citric Acid (% Citric Acid).

Both the analyses were performed in triplicate for each plot, and results are expressed as mean value for each genotype ± standard error.

Fruit Phytochemical Parameters

Fruit extraction method

The fruit phytochemical quality was analysed on blueberry samples from the bulked frozen fruits. Fruit samples were extracted by using the procedure described by Balducci *et al.* [17]. Briefly, for each plot, 10 g of chopped blueberries were weighed in a Falcon tube and the extraction started adding 20 ml of methanol, followed by a homogenization with the Ultra-Turrax T 25 (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The suspension was then agitated in the dark continuously for 30 min at room. After 10 min of centrifugation at 4500x*g* (Heraeus Megafuge 16, Thermo Fisher Scientific, Waltham, USA) the supernatant was collected into amber vials. The pellet was subjected to a second extraction identical to the first one, resuspending it in further 20 ml of methanol. The obtained supernatant was merged with the supernatant from the first extraction. The extracts were stored in the amber vials at – 20 °C until analysis. This extract was used for the analyses of Total Antioxidant Capacity, Total Phenol Content, Total Anthocyanins Content, and phenolic acids content.

For the vitamin C extraction, the method described by Zhong *et al*. [18] was adopted. Briefly, for each plot, 1 gram of frozen chopped blueberries was put in a 50 ml Falcon® tube together with 4 ml of the extracting solution (5% metaphosphoric acid and 1 mM diethylenetriaminepentaacetic acid “DTPA”). After homogenisation with Ultra-Turrax T 25 (IKA®-Werke GmbH & Co. KG, Staufen, Germany) and 5 min in

an ultrasonic bath (Transsonic 470, Elma GmbH, Singen, DE), the samples were centrifuged at 980x*g* for 10 min at 4°C (Heraeus Megafuge 16, Thermo Fisher Scientific, Waltham, USA). After filtering the supernatant with 0.45 µm sterile nylon filter (ReliaPrep Syringe filters, Ahlstrom, Bärenstein, DE), the samples were kept in 1.5 ml Eppendorf tubes at -20°C until analysis.

Total Antioxidant Capacity

The fruit total antioxidant capacity (TAC) was evaluated using the reduction of ferric tripyridyltriazine (Fe^{+3} –TPTZ) – FRAP method [19], with some modifications [20]. Briefly, the FRAP reagent solution was freshly prepared immediately prior to procedure, by combining ten volumes of sodium acetate (300 mM, pH 3.6) with one volume of TPTZ (10 mM in HCl 40 mM) and one volume of ferric chloride (20 mM) aqueous solutions. Then, 100 µL of sample (blank/Trolox standard/10-fold milliQ water diluted blueberry methanolic extract) were added to 900 µL of FRAP reagent. The mixture was quickly vortexed for 15 seconds and allowed to react 4 minutes. Then, the absorbance of the solution was read at 593 nm with a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) against blank. Trolox aqueous dilutions were used for calibration. Results were expressed as micro moles of Trolox equivalents per gram of fresh weight (µMTE g^{-1} FW). The analysis was performed in triplicate for each plot, and results are expressed as mean value for each genotype ± standard error.

Total Phenol Content

The fruit total phenol content (TPH) was evaluated on fruit extracts according to the Folin-Ciocalteu reagent method [21] and quantified with Gallic Acid as standard. Briefly, a glass test-tube was filled with 7.0 mL water. Afterwards, 1 mL of the diluted sample (1:20) was added, followed by the addition of 500 mL Folin-Ciocalteu-Reagent and vortexing. After 3 min, 1.5 mL sodium carbonate (0.53 mol/L) was added, and the tube was mixed once more and then stored in the dark for 60 min. The absorbance of the sample was then measured at 760 nm. The data were calculated and expressed as mg gallic acid per kg fresh fruit (mg GA kg^{-1} FW). The analysis was performed in triplicate for each plot, and results are expressed as mean value for each genotype ± standard error.

Total Anthocyanin Content

The fruit total anthocyanin content (ACY) was measured using the pH differential shift method [22]. Briefly, the methanolic extracts were diluted to a ratio of 1:10 with potassium chloride (pH 1.00) and with sodium acetate (pH 4.50), and then the corresponding maximum absorbances for both of the solutions were measured at 500 nm and at 700 nm. The data were expressed as mg of cyanidin-3-glucoside per kg of fresh

185 weight (mg CYA-3-GLU kg^{-1} FW). The analysis was performed in triplicate for each plot, and results are
186 expressed as mean value for each genotype ± standard error.

187 Phenolic acids Content

188 The amount of the main phenolic acids present in blueberries was determined through HPLC methodology,
189 as described by Fredericks *et al.* [23], with some modifications. The HPLC system comprised a Jasco PU-
190 2089 Plus (Jasco, Easton, MD, USA) controller with a flow rate set at 1.0 mL min^{-1}, a Jasco UV-2070 Plus
191 ultraviolet (UV) detector (Jasco, Easton, MD, USA) set at absorbance of 320 nm, and a column Aqua Luna
192 C18 250×4.6 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 2% (v/v) acetic acid
193 in Milli-Q water (eluent A) and of acetic acid in water and acetonitrile (1:49:50, v/v/v; eluent B). The
194 gradient program was as follows: 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10%
195 B (1 min), and 10% B for 5 min to re-equilibrate the column. The phenolic acids were quantified using
196 external chlorogenic acid and caffeic acid calibration curves. Values were calculated and expressed as mg
197 of the corresponding phenolic acid per kg of fresh weight (mg kg^{-1} FW). The analysis was performed in
198 triplicate for each plot, and results are expressed as mean value for each genotype ± standard error.

199 Vitamin C Content

200 Vitamin C content was measured according to the method of Helsper *et al.* [24], with some modifications.
201 The blueberry extracts previously obtained were subjected to HPLC analysis. The instrument consists of a
202 Jasco PU-2089 Plus pump (Jasco, Easton, MD, USA), flow rate 0.5 mL min^{-1}, equipped with a Jasco UV-
203 2070 Plus ultraviolet (UV) detector (Jasco, Easton, MD, USA) set at absorbances of 244 nm, and a column
204 Supelcosil LC8 150×4.6 mm (Supelco, Saint Louis, MO, USA). Quantification of the vitamin C content
205 was carried out through a calibration curve prepared by running standard concentrations of vitamin C.
206 Results are expressed as mg vitamin C per kg fresh weight (mg kg^{-1} FW). The analysis was performed in
207 triplicate for each plot, and results are expressed as mean value for each genotype ± standard error.

208 **Statistical analyses**

209 The fruit productive, qualitative, and phytochemical parameters were analysed in triplicate for each sample.
210 Data were analysed using one-way analysis of variance (ANOVA), with each genotype and years as an
211 independent variable. Significant differences within genotypes were calculated according to Duncan tests,
212 and differences at $p<0.05$ were considered as significant. Correlations among the productive (plant yield
213 and average fruit weight), qualitative (SSC and TA) and phytochemical (TAC, TPH, ACY, phenolic acids
214 and vitamin C) parameters were analyzed using Pearson's correlations ($p<0.01$ and $p<0.05$). Principal

215 Component Analysis (PCA) was also used to evaluate the levels of association among the productive,
216 qualitative and phytochemical parameters, and among the evaluated genotypes. The two most significant
217 factor loading values >0.4 were used to identify the most important variables and observations in each
218 dimension (PC). The factor loading values are the correlations of each parameter with the PC. They are
219 represented as vectors (positions) in the space represented by the axes of the PCA bi-plot. In the graphs,
220 the parameters and the genotypes that are closest to each other in the same geometric plane of the bi-plot
221 are considered as interrelated, and consequently the parameters and the genotypes that are distant to each
222 other are not related or negatively related. The greater the distance of a vector from the origin of the axis,
223 the higher the correlation of the variable with the PC represented in that dimension (axis). All the analyses
224 were performed with the software *Statistica 7* (StatSoft, TIBCO Software, Palo Alto, CA, USA).
225
226 **RESULTS AND DISCUSSION**
227 **Fruit harvest**
228 According to first harvest date detected in the years 2014 and 2015, it is possible to divide the blueberry
229 genotypes into three groups: early, mid and late ripening time, as showed in Tables 1 and 2. Genotypes
230 belonging to the different ripening times maintain their tendency to ripen sooner or later in both years of
231 study. These data are confirmed by the GDDs values, which showed lower values for the early ripening
232 genotypes and increased in the mid and late ripening genotypes. The only exception could be considered
233 'Patriot', which belong to the early genotypes in 2014, while it behaves as a mid-ripening time cultivar in
234 the year 2015. The PI is another fundamental value for the determination of the ripening time in blueberry
235 and, according to the mean values of PI for the two years, 'Patriot' results a mid-ripening cultivar.
236 As shown in Figure 2, the temperature trend in 2014 and 2015 years, from 1st January to late June, was
237 quite similar, even if there were some differences in specific moments of the year. As a result, the early
238 ripening cultivars ('Duke', 'Hortblue Petite', and 'Nui') slightly anticipate their first harvest date in the
239 year 2014. Contrarily, some mid ('Blueray', 'Early Blue' and 'Roxy Blue') and late-ripening genotypes
240 ('Blue Silk', 'Hortblue Poppins' and PFR075) slightly anticipated their first harvest in 2015 in respect to
241 2014 (Table 1).
242 The total average yield per plant and the AFW are also shown in Tables 1 and 2. The best AFW was
243 registered by 'Nui' in the year 2014, with 2.86 g/fruit, followed by 'Cosmopolitan' with 2.85 g/fruit.
244 'Cosmopolitan' also showed a very interesting AFW in the 2015, being the highest with 2.14 g/fruit.

Interestingly, this cultivar was the only to present an AFW higher than 2.00 g/fruit in the year 2015. In a previous study from our group [17], 'Cosmopolitan' (3.00 g/fruit) and 'Nui' (2.90 g/fruit) confirmed the big size of their fruits in the productive seasons 2012-2013, in the same pedoclimatic conditions of the present study.

All the other cultivars registered AFW values comprised between 1.00 and 1.99 g/fruit, except 'Bluecrop', 'Hortblue Petite' and 'Hortblue Poppins' which presented values of 0.99, 0.64 and 0.72 g/fruit, respectively (Table 1). This result underlines the importance of the cultivation year in determining the AFW. In fact, for each of the analyzed genotypes, the highest value of AFW was registered in 2014. Many genotypes performed for an AFW higher than 2.00 g/fruit in 2014, while only 'Hortblue Poppins' showed a value lower than 1.00 (0.97 g/fruit). A different behavior of genotypes along years (1998-2007) has been also reported by Ehlenfeldt and Martin [25], with fruit weight of 'Duke' ranging from 1.2 to 2.3 g/fruit, while 'Bluecrop' showed values comprised between 1.3 and 1.9 g/fruit. This genotype showed a similar range also in Wach [26], with fruits ranging from 1.73 to 1.98 g (years 1996-1999).

Differently from the AFW, the plant total yield varied in the years depending on the genotypes. In fact, some genotypes showed higher values of total yield in 2014, while other showed higher values in 2015. As an example, the selection PFR005 showed the highest yield in 2014 with 1433 g/plant, while in 2015 it showed a lowest yield (984 g/plant). In the biennium 2012-2013, it showed an average value of 1160 g/plant [17]. Contrarily, the cultivar 'Hortblue Petite' showed the highest plant yield in 2015 with 1503 g/plant, while in 2014 it produced only 833 g/plant; it even presented a very low mean value in the biennium 2012-2013 in the same pedoclimatic conditions (493 g/plant) [17]. In Wach [26], 'Bluecrop' also showed a great variability of plant yield among years (0.71-3.5 kg/bush), and in Ehlenfeldt and Martin [25] it showed again great variability among years but with higher yields (3.7-7 kg/plants), as well as for 'Duke' (3.5-7.4 kg/plant).

Fruit quality parameters

Figure 3 shows the mean values of fruit SSC in the two years of analysis. The best genotype for this parameter resulted PFR075, which produced fruits with the highest two-years mean value (13.8 °Brix) of SSC (Figure 4), followed by fruit of 'Blue Silk' (13.1 °Brix). From a statistical point of view, mean values of both genotypes resulted similar to all the genotypes with more than 12 °Brix, such as 'Hortblue Petite', 'Nui', 'Blue Ray', 'Patriot', 'Blue Silk' and 'Hortblue Poppins', then followed by the other genotypes having fruit SSC values above 10 °Brix.

Regarding fruit TA, it is well-known that high values of TA can have a negative incidence in the sensorial perception of the blueberry fruit by the consumer. Lower values of TA could better balance the sugar/acid ratio of blueberry, increasing the acceptance by the consumer. On the light of this, Figure 3 showed that 'Blueray' produced the less acidic fruits among the studied genotypes, presenting the two years mean value of 0.93 % Citric Acid. The fruit of 'Early Blue' also presented a low TA value, below the 1 % of Citric Acid (0.98).

Even if the statistical analysis did not show many significant differences, it is possible to divide the analyzed genotypes into low fruit TA (from 0.93 % Citric Acid in 'Blueray' to 1.05 % Citric Acid in PFR005 and 'Roxy Blue'), medium fruit TA (from 1.20 % Citric Acid in 'Patriot' to 1.30 % Citric Acid in 'Hortblue Petite'), and high fruit TA (from 1.41 % Citric Acid in PFR075 to 1.59 % Citric Acid in 'Nui').

Fruit phytochemical parameters

Phytochemical parameters represent the healthfulness of the analyzed fruits, indicating the potential positive impact of blueberry consumption on the health of the final consumer. It is possible to divide the fruit phytochemical parameters analyzed in this study in two different groups. The first belongs to the spectrophotometrically detected parameters, and comprises the TAC, TPH and ACY evaluation. The second one comprises the parameters evaluated through the utilization of the High-Performance Liquid Chromatography (HPLC) technique, in particular phenolic acids and vitamin C content.

Spectrophotometric analyses

In past decades, much attention has been given to the total antioxidant capacity (TAC) of foods as an eligible parameter for quality and as an indicator of beneficial bioactive compounds present in foods and of their capacity to attenuate the incidence of several chronic pathologies.

According to the ANOVA analysis, the year, the genotype, and the year x genotype effects were significant in the determination of fruit TAC values (Table 3). For this trait, the PFR075 fruit stands out from all the other genotypes, resulting with the highest two-years TAC mean value (25.76 uM TE g^{-1} FW), and demonstrating an improvement in the breeding program toward the healthiness of blueberry fruits.

For giving an idea about the difference between PFR075 and the other genotypes, it is to mention that the other genotypes with high TAC fruit values were 'Hortblue Petite' and 'Hortblue Poppins', respectively with 19.98 uM TE g^{-1} FW and with 19.88 uM TE g^{-1} FW. The fruit of 'Cosmopolitan' resulted with the lowest TAC value (14.33 uM TE g^{-1} FW, Table 3).

However, TAC values obtained in the present study could be considered very high in respect to other studies found in literature. In Okan *et al*. [27], many genotypes cultivated in different provinces of Turkey resulted with lower TAC values of the fruit than in our study, measured with the same FRAP method: 'Bluecrop' (4.55-7.71 uM Trolox g^{-1} FW), 'Blueray' (9.86 uM Trolox g^{-1} FW), 'Early Blue' (9.54 uM Trolox g^{-1} FW), and 'Patriot' (12.87 uM Trolox g^{-1} FW) presented almost half of the content measured in our study; only 'Duke' (22.45 uM Trolox g^{-1} FW) showed a better value than in our study (17.37 uM Trolox g^{-1} FW).

The antioxidant capacity of blueberries could be ascribed to the phenolic phytochemicals present in these fruits [28]. As a demonstration, the highest value of TPH was shown by PFR075 (3778.4 mg GA kg^{-1} FW), which showed also the highest value of TAC. This result confirmed the optimal performance of PFR075 demonstrated in the previous biennium [17], when it showed a very high TAC value and a good TPH value (2891 mg GA kg^{-1} FW).

Also 'Hortblue Poppins' fruits showed a high value of TPH (3115.6 mg GA kg^{-1} FW), confirming the correspondence with the high FRAP value of the fruit. Furthermore, fruits of 'Cosmopolitan' and 'Roxy Blue' showed the lowest values both for TPH (1594.3 and 1936.0 mg GA kg^{-1} FW, respectively) and TAC (14.33 and 16.95 µM TE g^{-1} FW, respectively), confirming the relation between TAC and TPH, and showing the low interest of these two genotypes for the phytochemical content in the current environmental/cultivation conditions (Table 3). However, those two genotypes showed slightly better results for TPH in the previous biennium, in particular 'Roxy Blue', with 2186 mg GA kg^{-1} FW [17]. Those findings confirm that, as for the TAC, also for the TPH the year, the genotype, and the year x genotype interactions are significant.

As for the TAC values, TPH results of our study differ from what achieved by Okan *et al.* [27]. The fruits of four genotypes over five in common with our study registered a TPH value lower than what we detected for 'Cosmopolitan', which was the worst. This difference can confirm the important role of environment/cultivation conditions on blueberry fruits TAC value and TPH content.

The anthocyanin compounds represent the biggest group of water-soluble natural pigments and belong to the flavonoids class, which in turn are the main phenolic representative class, as well as the main group of phenolic assumed with the diet [29]. As expected, PFR075 fruit, which possessed the highest values for TAC and TPH, confirmed its interest also for the highest total content of ACY (1908.8 mg CYA-3-GLU kg^{-1} FW), followed again by 'Hortblue Poppins' (1566.8 mg CYA-3-GLU kg^{-1} FW), which in turn showed high values of both TAC and TPH.

Similar ranges of ACY concentrations were found in literature for highbush blueberries [30,31], while in the previous biennium, we have found slightly lower values [17]. This latter study, however, confirmed PFR075 as the best genotype for ACY content also in the biennium 2012-2013, with an average value of 1744 mg CYA-3-GLU kg^{-1} FW.

Beyond the high values, also the low values of ACY were related to the other phytochemical parameters: in fact, genotypes like 'Blue Silk', 'Duke', and in particular 'Cosmopolitan', together with the lowest values of TAC and TPH, showed also very low values for ACY, with 1109.0, 1110.4, and 1056.9 mg CYA-3-GLU kg^{-1} FW respectively (Table 3). However, for this parameter, the worst genotype resulted 'Bluecrop' with a value of 822.5 mg CYA-3-GLU kg^{-1} FW. This genotype confirmed a very low value of ACY in Okan *et al.* [27], with only about 500 mg CYA-3-GLU kg^{-1} FW. However, in Rodriguez-Mateos *et al.* [30], 'Bluecrop' resulted the best genotype for fruit ACY content (1873 mg kg^{-1} FW), but it is known that the concentrations of this class of compounds could be influenced by many factors, as extraction and analytical methods, fruit ripening and the genotype and the pedoclimatic conditions [37].

Chromatographic analyses (HPLC)

Phytochemicals measured through HPLC comprised phenolic acids and vitamin C content. Regarding the first group of compounds, chlorogenic acid is one of the most prominent phenolic acids in blueberries [11]. 'Bluecrop' results the richest cultivar of chlorogenic acid for the years 2014 and 2015 (1581 mg kg^{-1} FW) (Table 4), differently from what observed by Rodriguez-Mateos *et al.* [30], where 'Bluecrop' fruit showed the lowest content of chlorogenic acid (about 400 mg kg^{-1} FW) among six highbush blueberry varieties from UK and a lowbush blueberry variety from North America. Together with 'Bluecrop', also 'Blueray' and PFR075 showed high levels of fruit chlorogenic acid content (1401 and 1395 mg kg^{-1} FW, respectively), while fruit of 'Cosmopolitan', 'Hortblue Petite' and in particular 'Hortblue Poppins', revealed the lowest values of chlorogenic acid (790, 658, and 321 mg kg^{-1} FW respectively) (Table 4). The range of chlorogenic acid fruit content measured in our study agrees with the results found by Ochmian *et al.* [31] but resulted higher than Yousef *et al.* [33] and Okan *et al.* [27].

Regarding the caffeic acid, its presence in the studied blueberry genotypes was scarce, even if its biological activities are effective also at small amounts. 'Nui' and again PFR075 were the genotypes with the highest concentrations of this phenolic acid (246 and 182 mg kg^{-1} FW, respectively), while 'Roxy Blue', 'Blue Silk' and 'Hortblue Poppins' registered values of caffeic acid lower than 10 mg kg^{-1} FW (7.7, 6.8, and 6.3

mg kg^{-1} FW respectively) (Table 4). Yousef *et al.* [33], and especially Okan *et al*. [27], found values of caffeic acid even lower than our study, with some genotypes that did not reveal any trace of caffeic acid.

Regarding fruit vitamin C content, only few studies assessed the amount of this compound in blueberries. In this research, fruit of 'Hortblue Petite' had the highest mean value of vitamin C (43.2 mg kg^{-1} FW), followed by 'Nui' (36.2 mg kg^{-1} FW) and 'Bluecrop' (33.4 mg kg^{-1} FW) (Figure 4), while the lowest fruit content was detected for PFR005 (24.0 mg kg^{-1} FW), 'Hortblue Poppins' (23.5 mg kg^{-1} FW) and 'Blue Silk' (20.4 mg kg^{-1} FW) (Figure 4). In general, vitamin C values in our study resulted slightly lower than mean values reported for highbush blueberries in Prior *et al*. [13] and Starast *et al.* [34]. In the first study, it was reported that highbush blueberries showed an average value of 72 mg kg^{-1} FW, while in the second one the half-highbush blueberry showed a content of vitamin C of 150 mg kg^{-1} FW. One of the reasons of this difference could be that, besides the genotype and environmental effect, the cracking of blueberry fruit skin could lead to the oxidation of ascorbate, resulting in a significant decrease of its concentration [13].

Correlation matrix

Fruit TAC (measured with FRAP method), TPH and ACY, two-years mean values, resulted strongly correlated each other ($p<0.01$), giving that TAC is strictly related to antioxidant activity induced by high concentration of phenols and anthocyanins, among other phytochemical compounds in blueberries fruits (Table 5). The same strong correlation ($p<0.01$) among FRAP, TPH and ACY was also detected by Okan *et al*. [27].

However, TAC did not result correlated to phenolic acids and vitamin C, even if those molecules are known as strong antioxidant compounds. This is probably due to the low concentration of caffeic acid and vitamin C accumulated in blueberry fruit. Furthermore, chlorogenic acid, even if is present in higher quantity, is not correlated to TAC.

TAC, TPH (both at $p<0.01$) and ACY ($p<0.05$) content resulted strongly related to the SSC, which means that sweeter fruits seems to be richer of bioactive compounds belonging to phenolic category (Table 5). However, SSC was not related to phenolic acids and vitamin C. SSC was also inversely related to AFW ($p<0.05$). Those smaller fruits could also result healthier for the human consumption, giving that the AFW is strongly inversely related to TAC ($p<0.01$). AFW is correlated with caffeic acid, meaning that bigger fruits contain higher amount of this phytochemical ($p<0.05$).

Caffeic acid, chlorogenic acid, and vitamin C resulted correlated each other. In particular, caffeic acid resulted strongly correlated with vitamin C concentration ($p<0.01$). Caffeic acid ($p<0.01$) and vitamin C

392 ($p<0.05$) resulted also inversely correlated with PI, meaning that higher biosynthesis of those compounds
393 could be stimulated in early-ripening blueberries, probably also associated to the milder climatic conditions
394 of early-ripening period (Figure 2). In fact, early ripening genotypes ('Nui', 'Hortblue Petite', 'Duke' and
395 'Patriot') were among the best genotypes for caffeic acid and vitamin C content (Table 4 and Figure 4). PI
396 resulted also inversely correlated to plant yield ($p<0.05$), indicating that early ripening genotypes tend to
397 be more productive than the late ripening genotypes. Similarly, this correspondence is evidenced in Table
398 2, where all the early ripening genotypes registered an average total production for the years 2014 and 2015
399 higher than all the late ripening genotypes. PI is also strongly correlated with TPH ($p<0.01$), meaning that
400 late ripening genotypes ('Bluecrop', 'Hortblue Poppins' and in particular PFR075) showed the highest
401 values of fruit TPH content (Table 3). On the light of these last considerations, it resulted that TPH was
402 strongly inversely correlated with plant yield ($p<0.01$) (Table 4).

403 **PCA**

404 The PCA bi-plot of productive, qualitative and phytochemical parameters showed interesting results,
405 highlighting a common trend for some of the analyzed parameters (Figure 5). The spectrophotometric
406 phytochemical parameters result in the same quadrant (higher left), together with the SSC content. The
407 interaction among phytochemical parameters and SSC content has been also evidenced by the correlation
408 matrix (Table 5). Similarly, AFW and plant yield relied on the lower right quadrant, opposite to the
409 phytochemicals and SSC quadrant. This means that among the phytochemicals (and SSC) parameters and
410 the productive parameters (AFW and plant yield) there is no relation or negative relation, as demonstrated
411 also by the correlation matrix (Table 5). Finally, it is interesting to note that all the HPLC-measured
412 compounds (chlorogenic acid, caffeic acid and vitamin C) are in the same lower left quadrant, so they are
413 related each other, as also suggested by the correlation matrix. Also the TA vector is placed in this quadrant
414 indicating that the TA of fruits could be related to the presence of those three acid compounds (chlorogenic,
415 caffeic or ascorbic acid).

416 Regarding the distribution of the genotypes on the bi-plot plan, it is possible to note some interesting results
417 (Figure 6). First of all, all the points belonging to the PFR075 genotype are located in the left part of the
418 graph, corresponding to the high content of TAC, TPH, ACY and SSC. As previously stated, this genotype
419 was effectively interesting for the high amount of those compounds. Then, 'Cosmopolitan' is concentrated
420 in the right part of the graph, close to the vectors of plant yield and AFW. 'Hortblue Poppins', which
421 resulted one of the latest genotypes, presents its points in the upper-left part of the graph, in correspondence

with the PI value. Furthermore, together with 'Early Blue' cultivar, it presents all its points in the same quadrant of the phytochemical compounds' vectors, confirming the interesting nutritional features already evidenced in Table 3.

CONCLUSION

This study demonstrated that by using new breeding material it is possible to generate new genotypes with higher adaptability to Mediterranean hot summer climate conditions in the mid-Adriatic area. The new blueberry genotypes tested have shown contrasting results for the productive parameters: PFR005 showed the higher average plant yield in the biennium of study, without an outstanding AFW, while PFR075 showed the lowest plant yield among all the studied genotypes, but with an AFW among the best in the biennium, being the highest for the late-ripening genotypes. This selection showed fruits with the highest SSC value that, combined with a medium-high TA value, gives a good sugar/acid ratio in the fruit. Regarding the fruit nutritional parameters, the breeding program reached a very high-quality level with the selection PFR075, resulting the best genotype, among all the tested blueberries, in terms of fruit TAC, TPH and ACY. Fruits of PFR005 selection also showed good nutritional values, in particular for TAC, even if at lesser extent than PFR075. For the HPLC analyses, PFR005 and mostly PFR075 showed very interesting values of fruit chlorogenic and caffeic acid content.

Some cultivars also demonstrated a good adaptability to cultivation in mid-Adriatic area (characterized by mild climate and chalky soils), with 'Cosmopolitan' and 'Nui' showing the highest AFW values, supported also by medium-high levels of plant yields. The most positive SSC/TA ratios were obtained for fruits of 'Blueray' and 'Hortblue Poppins', while high nutritional values were detected for 'Hortblue Poppins', 'Hortblue Petite' and 'Early Blue'. 'Hortblue Petite' resulted very interesting also for vitamin C content, showing the highest value, while 'Bluecrop' and 'Blueray' fruits possessed the highest values for chlorogenic acid.

The PCA analysis demonstrated high relation among parameters. As expected, the productive parameters (Plant yield and AFW) are close to each other in the bi-plot graph. Thus, confirming the importance to breed new blueberry cultivars with increased fruit size for increasing yield and reduce harvesting costs. The sensorial quality of the fruit expressed as SSC resulted related to TPH, ACY and TAC. This confirming the possibility to combine the high content of sensorial compounds, such as SSC, with health-related compounds such as TPH, ACY. Their correlation with TAC also confirms their role in determining the

antioxidant capacity of blueberry fruit combined with a higher sweetness that can be better appreciated by the consumer. On the contrary, TA is related to all the phytochemicals analyzed characterized by acid compounds, such as caffeic acid, chlorogenic acid and ascorbic acid. Therefore, blueberry fruit nutritional value determined with increased content of these compounds can result with a negative appreciation by the consumer for the increased acidity.

Comparing our results with what available in literature, is emerging that varieties tested in our conditions produced fruits with higher phytochemical content, in particular TPH associated with higher TAC values, of the same varieties tested with the same analytical methods in other cultivation conditions. It could be affirmed that our cultivating conditions, with warmer climate and soil pH inducing plant stress, promote higher content of antioxidant/nutritional compounds in blueberry fruits.

The application of these results to blueberry germplasm evaluation and tailored breeding program will help to create new resilient cultivars useful to expand blueberry cultivation in less optimal climatic and soil conditions.

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CONFLICT OF INTEREST

None.

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567

568 **FIGURE LEGENDS**

569 **Figure 1:** Evolution of the blueberry production area in the World in the last 20 years (Source FAOSTAT
570 http://www.fao.org/faostat/en/#data/QC/visualize).

571 **Figure 2:** Daily mean temperatures from 1st January to 24th June for the two years of study (2014 and
572 2015) at the experimental field, Agugliano (AN), Italy.

573 **Figure 3:** Mean Soluble Solids Content (SSC) and Titratable Acidity (TA) for the biennium (2014-2015)
574 of the blueberry genotypes. Genotypes are listed in order of ripening time, from earlier to later. Different
575 lowercase letters mean significant differences for SSC (Duncan test $p< 0.05$). Different uppercase letters
576 mean significant differences for TA (Duncan test $p< 0.05$). Data are shown as means ± standard errors
577 (n=6).

578 **Figure 4:** Mean vitamin C content for the biennium (2014-2015) of the blueberry genotypes. Genotypes
579 are listed in order of ripening time, from earlier to later. Different letters mean significant difference
580 (Duncan test $p< 0.05$). Data are shown as means ± standard errors (n=6).

581 **Figure 5**: Bi-plot of the productive, qualitative and phytochemical parameters analyzed in this study (vector
582 distribution). Factor 1 and Factor 2 explain 40.41% of the data variation.

583 **Figure 6**: Bi-plot of blueberry genotypes analyzed in this study (case distribution). Only the ten genotypes
584 with a clear grouping-distribution are indicated. Factor 1 and Factor 2 explain 40.41% of the data variation.

585

FIGURES

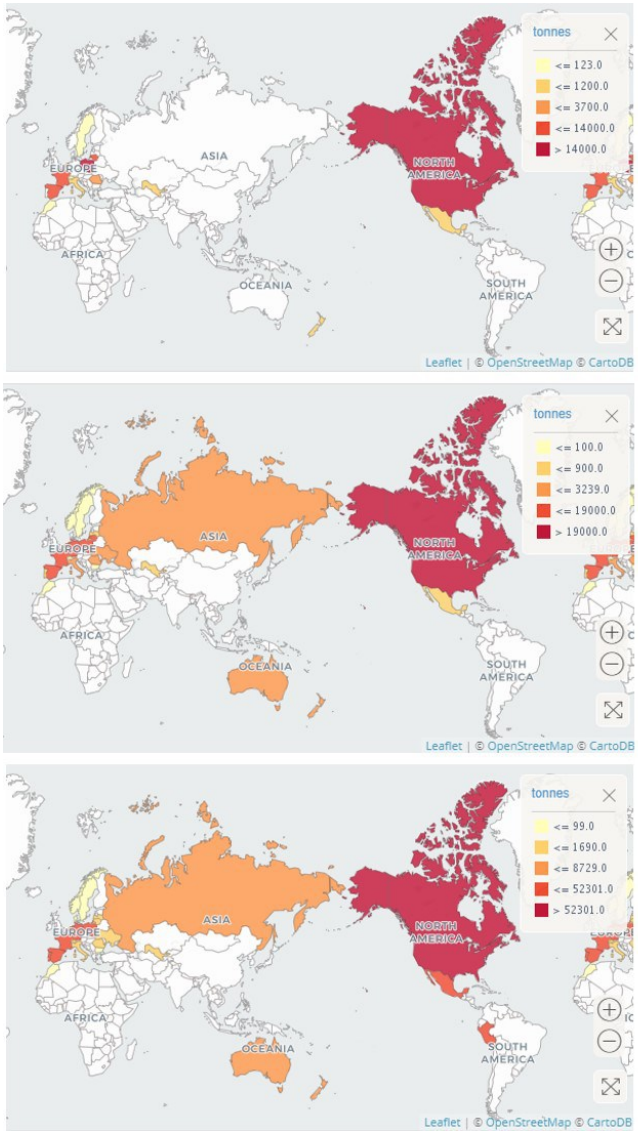
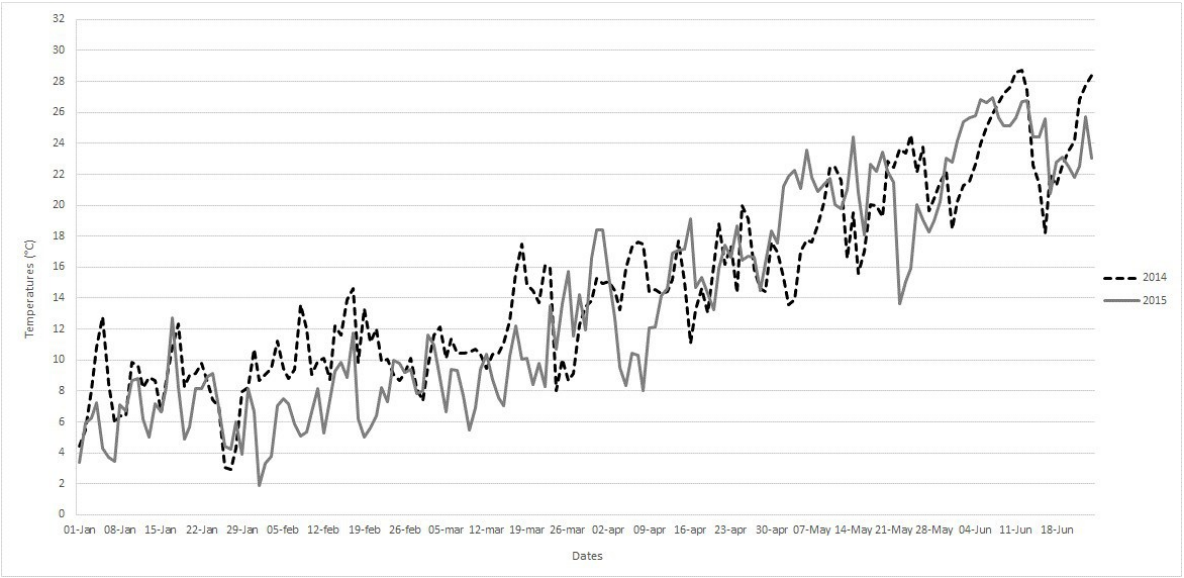


Figure 1

590 

591 **Figure 2**

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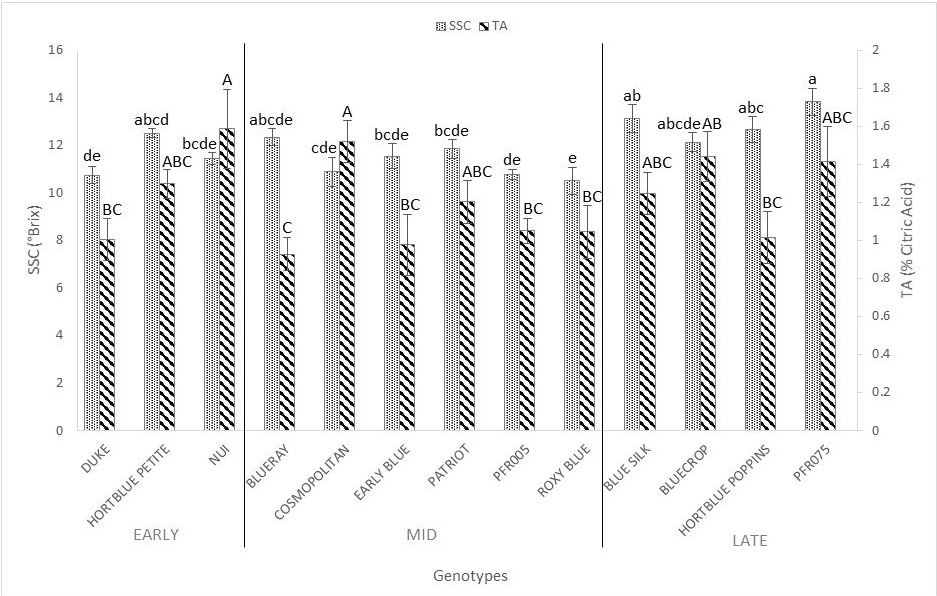

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594 **Figure 3**

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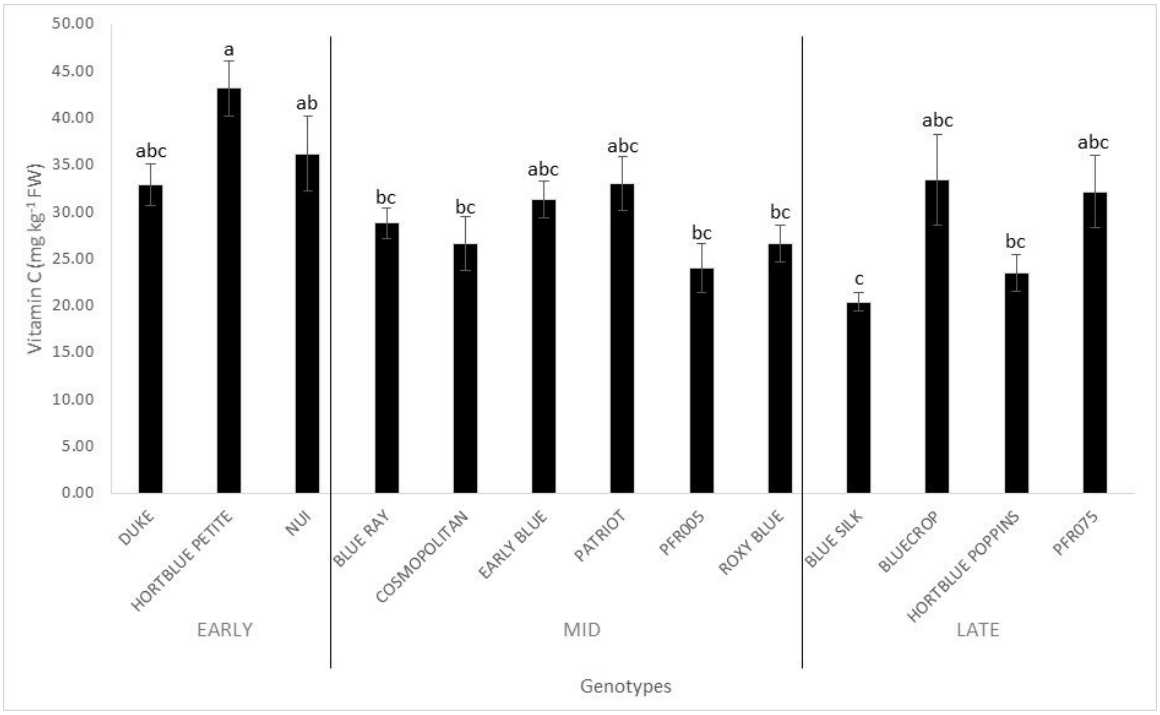

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597 **Figure 4**

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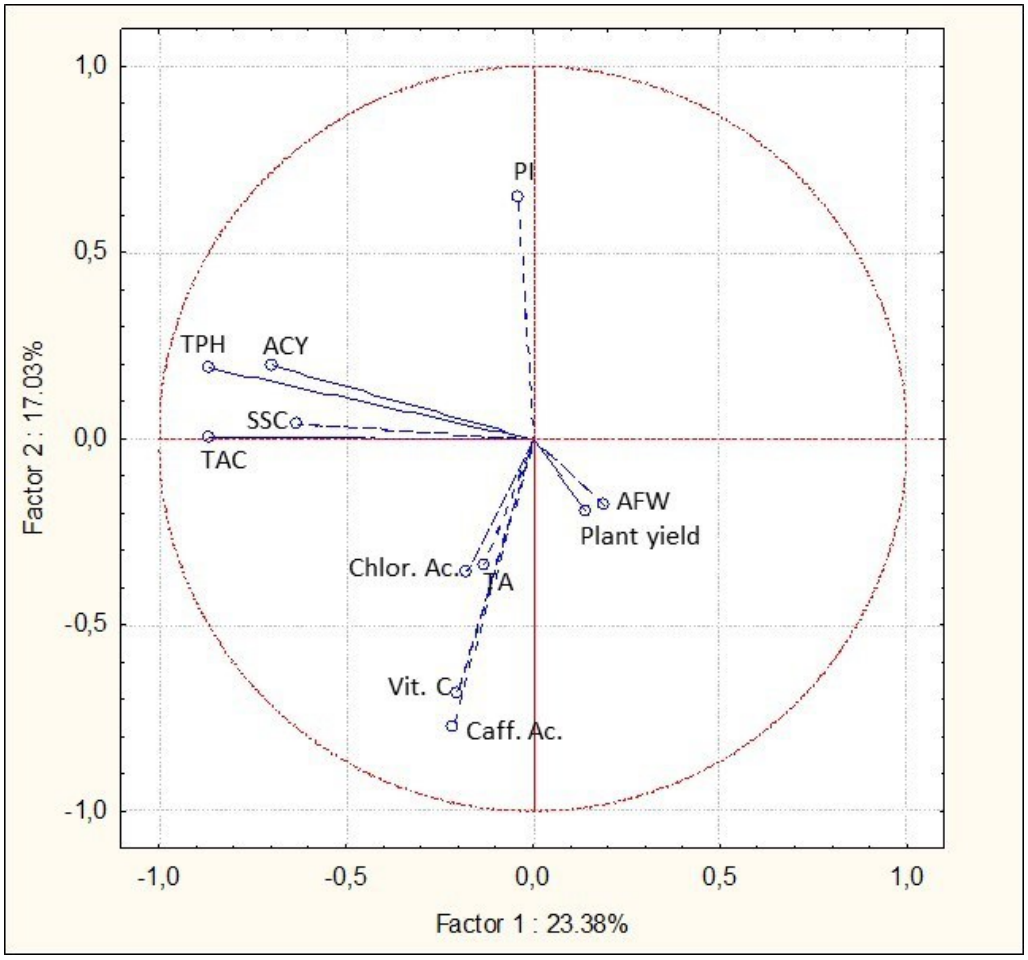
599 

600 **Figure 5**

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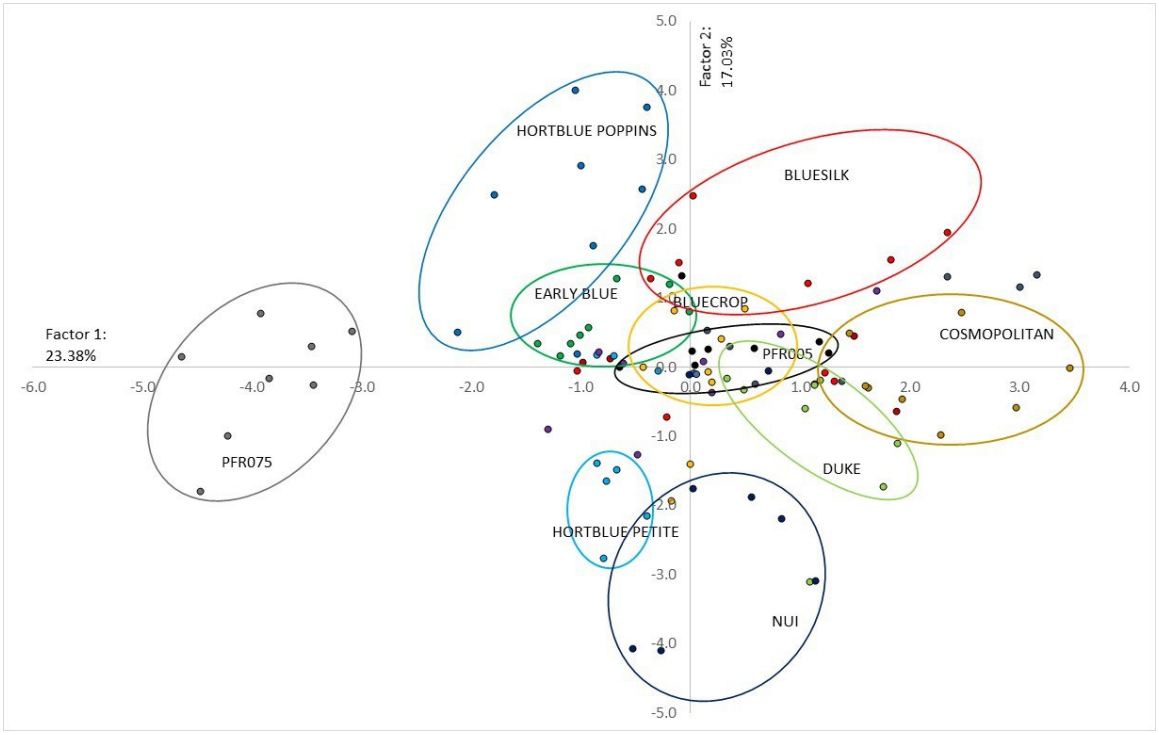
602 

603 **Figure 6**

604

TABLES

Table 1. First harvest data, Growing Degree Days (GDD), Precocity Index (PI), Average Fruit Weight (AFW), and Total Plant Yield for the years 2014 and 2015 of the blueberry genotypes. Genotypes are listed in order of ripening time, from earlier to later. Data are shown as means ± standard errors (n=10).

PRECOCITY	YEAR	GENOTYPE	FIRST HARVEST DATA	GDD (°C)	PI (days)	AFW (g/fruit)	PLANT YIELD (g/plant)
EARLY	2014	DUKE	3-Jun	1590	160.66 ± 0.11	1.87 ± 0.10	1170.61 ± 75.90
EARLY	2014	HORTBLUE PETITE	3-Jun	1590	155.87 ± 0.05	1.06 ± 0.06	832.68 ± 48.08
EARLY	2014	NUI	3-Jun	1590	160.48 ± 3.38	2.86 ± 0.19	954.77 ± 244.04
MID	2014	BLUERAY	13-Jun	1823	167.33 ± 4.09	2.33 ± 0.18	1069.17 ± 249.54
MID	2014	COSMOPOLITAN	9-Jun	1723	169.25 ± 0.45	2.85 ± 0.19	1242.54 ± 54.00
MID	2014	EARLY BLUE	13-Jun	1823	173.33 ± 5.26	1.38 ± 0.20	808.59 ± 97.77
MID	2014	PATRIOT	6-Jun	1652	167.95 ± 3.09	1.94 ± 0.17	1275.73 ± 12.48
MID	2014	PFR005	9-Jun	1723	170.35 ± 0.61	1.67 ± 0.02	1433.30 ± 12.18
MID	2014	ROXY BLUE	13-Jun	1823	171.80 ± 4.20	1.68 ± 0.17	993.19 ± 293.38
LATE	2014	BLUE SILK	17-Jun	1896	177.12 ± 1.33	2.00 ± 0.08	1146.33 ± 92.05
LATE	2014	BLUECROP	20-Jun	1954	181.17 ± 2.65	1.79 ± 0.21	529.10 ± 306.07
LATE	2014	HORTBLUE POPPINS	20-Jun	1954	183.59 ± 3.85	0.97 ± 0.14	680.94 ± 47.61
LATE	2014	PFR075	17-Jun	1896	172.90 ± 2.37	2.66 ± 0.22	762.26 ± 282.64
EARLY	2015	DUKE	4-Jun	1442	159.39 ± 0.87	1.08 ± 0.01	1168.00 ± 56.00
EARLY	2015	HORTBLUE PETITE	4-Jun	1442	159.74 ± 0.01	0.64 ± 0.01	1503.00 ± 205.00
EARLY	2015	NUI	6-Jun	1489	160.85 ± 1.54	1.34 ± 0.07	1237.00 ± 39.00
MID	2015	BLUERAY	11-Jun	1603	165.98 ± 0.25	1.38 ± 0.22	1325.50 ± 575.50
MID	2015	COSMOPOLITAN	9-Jun	1558	164.20 ± 1.09	2.14 ± 0.13	775.50 ± 66.50
MID	2015	EARLY BLUE	11-Jun	1603	166.04 ± 0.78	1.01 ± 0.08	933.00 ± 519.00
MID	2015	PATRIOT	9-Jun	1558	164.16 ± 2.47	1.00 ± 0.14	1112.33 ± 644.33
MID	2015	PFR005	9-Jun	1558	164.08 ± 4.21	1.26 ± 0.29	984.00 ± 380.00
MID	2015	ROXY BLUE	8-Jun	1536	162.61 ± 0.21	1.27 ± 0.04	680.00 ± 70.00
LATE	2015	BLUE SILK	16-Jun	1716	172.20 ± 1.13	1.15 ± 0.07	877.40 ± 119.40
LATE	2015	BLUECROP	20-Jun	1793	174.63 ± 2.75	0.99 ± 0.07	906.39 ± 298.99
LATE	2015	HORTBLUE POPPINS	16-Jun	1716	172.27 ± 1.25	0.72 ± 0.06	1264.00 ± 654.00
LATE	2015	PFR075	16-Jun	1716	171.68 ± 0.75	1.15 ± 0.07	621.00 ± 233.00

Table 2. Mean first harvest data, Growing Degree Days (GDD), Precocity Index (PI), Average Fruit Weight (AFW), and Total Plant Yield for the biennium (2014-2015) of the blueberry genotypes. Genotypes are listed in order of ripening time, from earlier to later. Data are shown as means ± standard errors (n=20).

PRECOCITY	GENOTYPE	FIRST HARVEST DATA	GDD (°C)	PI (days)	AFW (g/fruit)	PLANT YIELD (g/plant)
EARLY	DUKE	2-Jun	1516	160.03 ± 0.73	1.48 ± 0.33	1169.31 ± 54.47
EARLY	HORTBLUE PETITE	2-Jun	1516	157.81 ± 1.58	0.85 ± 0.18	1167.84 ± 299.44
EARLY	NUI	3-Jun	1540	160.67 ± 2.15	2.10 ± 0.63	1095.89 ± 183.40
MID	BLUERAY	11-Jun	1713	166.65 ± 2.43	1.86 ± 0.42	1197.34 ± 376.97
MID	COSMOPOLITAN	8-Jun	1641	166.72 ± 2.17	2.49 ± 0.32	1009.02 ± 196.98
MID	EARLY BLUE	11-Jun	1713	169.69 ± 4.28	1.19 ± 0.20	870.80 ± 309.12
MID	PATRIOT	6-Jun	1605	166.05 ± 2.76	1.47 ± 0.41	1194.03 ± 378.01
MID	PFR005	8-Jun	1641	167.21 ± 3.55	1.47 ± 0.24	1208.65 ± 286.05
MID	ROXY BLUE	9-Jun	1680	167.21 ± 4.47	1.47 ± 0.20	836.60 ± 216.04
LATE	BLUE SILK	15-Jun	1806	174.66 ± 2.25	1.57 ± 0.35	1011.86 ± 140.11
LATE	BLUECROP	19-Jun	1874	177.90 ± 3.47	1.39 ± 0.35	717.74 ± 291.12
LATE	HORTBLUE POPPINS	17-Jun	1835	177.93 ± 5.18	0.84 ± 0.14	972.47 ± 447.20
LATE	PFR075	15-Jun	1806	172.29 ± 1.52	1.91 ± 0.63	691.63 ± 219.20

613 **Table 3**. Mean Total Antioxidant Capacity (TAC), Total Phenol Content (TPH), and Total Anthocyanin Content (ACY) for the biennium (2014-2015) of the blueberry
614 genotypes. Genotypes are listed in order of ripening time, from earlier to later. Different letters mean significant difference (Duncan test $p < 0.05$). Data are shown as means ±
615 standard errors (n=6).

PRECOCITY	GENOTYPE	TAC (μMTE g^{-1} FW)	TPH (mgGA kg^{-1} FW)	ACY (mg CYA-3-GLU kg^{-1} FW)
EARLY	DUKE	17.37 ± 1.03^{ef}	1942.17 ± 58.76^{l}	1110.40 ± 29.55^{i}
EARLY	HORTBLUE PETITE	19.98 ± 0.55^{b}	2409.17 ± 192.62^{g}	1371.65 ± 6.83^{d}
EARLY	NUI	17.62 ± 1.00^{def}	2197.45 ± 31.67^{h}	1195.64 ± 9.49^{f}
MID	BLUERAY	17.81 ± 0.72^{def}	2493.23 ± 171.01^{fg}	1089.81 ± 70.03^{l}
MID	COSMOPOLITAN	14.33 ± 0.30^{g}	1594.30 ± 65.32^{m}	1056.89 ± 16.53^{m}
MID	EARLY BLUE	19.12 ± 1.08^{bc}	2944.98 ± 58.49^{c}	1534.13 ± 41.28^{c}
MID	PATRIOT	18.19 ± 0.97^{de}	2574.83 ± 38.15^{ef}	1317.80 ± 23.00^{e}
MID	PFR005	19.22 ± 0.86^{bc}	2658.96 ± 70.01^{e}	1160.56 ± 40.49^{g}
MID	ROXY BLUE	16.95 ± 1.14^{f}	1936.04 ± 125.16^{l}	1142.82 ± 21.98^{h}
LATE	BLUE SILK	17.11 ± 0.94^{f}	2087.59 ± 117.72^{i}	1109.00 ± 16.10^{i}
LATE	BLUECROP	18.58 ± 0.90^{cd}	2764.47 ± 97.64^{d}	822.45 ± 17.15^{n}
LATE	HORTBLUE POPPINS	19.88 ± 0.29^{b}	3115.59 ± 129.10^{b}	1566.77 ± 44.75^{b}
LATE	PFR075	25.76 ± 0.28^{a}	3778.38 ± 82.57^{a}	1908.78 ± 114.72^{a}

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Table 4. Mean chlorogenic acid and caffeic acid content for the biennium (2014-2015) of the blueberry genotypes. Genotypes are listed in order of ripening time, from earlier to later. Different letters mean significant difference (Duncan test $p< 0.05$). Data are shown as means ± standard errors (n=6).

PRECOCITY	GENOTYPE	CHLOROGENIC ACID (mg kg^{-1} FW)	CAFFEIC ACID (mg kg^{-1} FW)
EARLY	DUKE	1248.5 ± 34.2^{abc}	14.9 ± 3.3^{bcd}
EARLY	HORTBLUE PETITE	658.4 ± 27.5^{d}	16.0 ± 0.8^{bc}
EARLY	NUI	1134.0 ± 86.2^{abcd}	24.6 ± 0.6^{a}
MID	BLUERAY	1400.8 ± 144.5^{ab}	12.0 ± 0.2^{cdef}
MID	COSMOPOLITAN	789.9 ± 25.9^{cd}	10.3 ± 0.6^{defg}
MID	EARLY BLUE	1145.6 ± 32.0^{abcd}	10.1 ± 0.7^{defg}
MID	PATRIOT	1007.5 ± 176.5^{bcd}	11.2 ± 2.1^{cdefg}
MID	PFR005	1100.5 ± 126.2^{abcd}	12.6 ± 1.1^{cde}
MID	ROXY BLUE	1075.1 ± 165.1^{abcd}	7.7 ± 1.0^{efg}
LATE	BLUE SILK	911.5 ± 155.6^{bcd}	6.8 ± 0.8^{fg}
LATE	BLUECROP	1581.3 ± 129.7^{a}	13 ± 0.5^{cde}
LATE	HORTBLUE POPPINS	321.4 ± 30.8^{e}	6.3 ± 0.5^{g}
LATE	PFR075	1395.3 ± 161.8^{ab}	18.2 ± 1.0^{b}

Table 5: Pearson's correlation matrix of the fruit productive, qualitative and phytochemical parameters. *, **, correlation levels significant at p≤0.05 and p≤0.01 respectively. Red asterisks represent a positive correlation, while black asterisks represent a negative correlation. n.s. = not significant.

PARAMETERS	AFW	Pl. Yield	SSC	TA	TAC	TPH	ACY	Chlor. Ac.	Caff. Ac.	Vit. C
PI	n.s.	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	*
AFW		n.s.	*	n.s.	**	n.s.	n.s.	n.s.	*	n.s.
Pl. Yield			n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.
SSC				n.s.	**	**	*	n.s.	n.s.	n.s.
TA					n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TAC						**	**	n.s.	n.s.	n.s.
TPH							**	n.s.	n.s.	n.s.
ACY								n.s.	n.s.	n.s.
Chlor. Ac.									*	n.s.
Caff. Ac.										**