

Polyphenol compounds and other quality traits in blueberry cultivars

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Abstract.

BACKGROUND: Blueberry fruit available in the market comes from cultivars that have been selected for specific traits and not necessarily for high concentrations of health-promoting phytochemicals in the fruit.

OBJECTIVE: To identify and quantify the total and individual phenolic components and other quality traits from a combination of cultivars from two *Vaccinium* species (*V. corymbosum* and *V. virgatum*). The cultivar combination provided a continuous and extended fruit harvest.

METHODS: Fruit samples were collected from cultivars growing in a randomized complete block design. The phenolic components were assessed on fruit extracts, the fruit weight and firmness were assessed on fresh fruit and the rest of the traits were assessed on fruit juice.

RESULTS: For most traits the differences between *Vaccinium* species and cultivars were considerable. Strong and positive correlations were found between phenolic components and between fruit traits. For each cultivar the majority of the traits analysed in this work from a single year were highly correlated to the average across the three years of evaluation. Assessing traits from a single year of data should be mostly reliable for individual cultivars.

CONCLUSIONS: The combination of cultivars in this study was designed to offer the widest possible harvest window; however, it gives high variation in fruit quality.

Keywords: *Vaccinium corymbosum*, *Vaccinium virgatum*, cultivar, highbush blueberry, rabbiteye blueberry, polyphenols, fruit weights, fruit quality traits

1. Introduction

Cultivated blueberries (*Vaccinium corymbosum* and *V. virgatum*) are produced commercially in New Zealand and in recent years the total area planted with blueberries increased from 239 ha in 2000 to 700 ha in 2013 [1, 2]. *V. corymbosum* ($2n = 4x = 48$) is commercially known as the “highbush” blueberry and *V. virgatum* ($2n = 6x = 72$) is known as the “rabbiteye” blueberry. Within the *Vaccinium* species internationally, there is a large range of cultivars, but relatively few are available in New Zealand. The cultivars that are readily available from nurseries may come from overseas breeding programmes, where they have been selected for environments that may be considerably different from that in New Zealand.

Plant & Food Research (PFR) started a blueberry breeding programme in the 1980s with the aim of producing blueberry cultivars that were tailored to New Zealand conditions. Breeding objectives were specific agronomic and

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fruit traits such as high yield, disease resistance, winter chilling adaptability, seasonality range and hand-harvestable fruit of large size, good firmness and good flavour. A blueberry plantation of well-adapted cultivars has the potential to produce a good crop for many years and thus planting the right genotype is crucial. Growing two or more different cultivars in the same commercial block is a popular choice among New Zealand growers, to satisfy pollination requirements and to extend the production window. We studied a combination of cultivars that was designed to offer the longest possible combined harvest season for the North Island, Waikato Region of New Zealand. To make this possible, a mix of different *V. corymbosum* and *V. virgatum* cultivars was chosen, some of which had been released from the PFR blueberry breeding programme and others were comparator cultivars. The combination of cultivars allowed a five-month continuous fruit harvest, starting in mid-November (late spring in New Zealand) and finishing around mid-April (mid-autumn).

Blueberry fruit available in the market comes from cultivars that have been selected for specific agronomic and fruit traits such as high yield and large fruit size and not necessarily for high concentrations of health-promoting phytochemicals in the fruit. For this reason, we believed it was important to measure the phytochemical content and composition of the fruit from the new cultivars and from the comparators and to discuss the results in this study.

Blueberry fruit are widely believed to be good for health because of their high content of polyphenolic compounds, in particular anthocyanins [3]. Anthocyanins provide blueberries with their characteristic colour and have been shown to contribute to the antioxidant capacity of berry fruit [4–10]. The health value of anthocyanins has been reviewed [11, 12], as well as antioxidant capacity [10, 13, 14]. Anthocyanins are reported to have a role in improving circulation [15], preventing stroke [16], providing benefits to vision [17, 18], and their anti-inflammatory and anti-oxidative effects have been extensively reported [19–22].

The anthocyanins present in blueberry are galactosides, glucosides and arabinosides of the anthocyanidins delphinidin, cyanidin, petunidin, peonidin and malvidin, and their concentrations vary greatly between genotypes [23, 24]. Additionally, these glycosides may also be acylated [25]. Blueberries are the richest sources of the more hydrophobic malvidins and petunidins among a wide selection of fruits and vegetables [26]. Blueberries are also rich in delphinidin-3-galactoside and petunidin-3-glucoside [27]. Research shows that there is interest in the anthocyanin content of blueberry fruit and that changes are to be expected between fruit of different cultivars and between seasons [23, 24, 28].

With an increased consumption of fresh blueberry fruit over the last decade, there have been specific breeding objectives aimed to improve the fruit quality acceptance as well as the health benefits of the berries. However there is little knowledge on how the phenolics correlate with other different quality traits in blueberry.

The objectives of this three-year study were to: (1) identify and quantify the total and individual phenolic components in fruit from seven *V. corymbosum* and seven *V. virgatum* cultivars (2) measure fruit quality traits such as fruit weight, diameter, firmness, soluble solid content and titratable acidity (3) investigate differences in phenolic components and fruit traits between the *Vaccinium* species and survey the homogeneity of fruit quality during the protracted harvest season (4) investigate the correlations between phenolics and fruit quality traits.

2. Materials and methods

2.1. Chemicals

Solvents and general chemicals were obtained from local suppliers. Reference standards were obtained as follows: cyanidin-3-glucoside from Extrasynthese (Genay, France), chlorogenic acid and rutin from Sigma-Aldrich.

2.2. Blueberry cultivation and fruit sample preparation

Blueberry fruit (*V. corymbosum* and *V. virgatum*) analyzed in this work were harvested when fully ripe from plants grown in test plots at the PFR – Ruakura Research Centre – New Zealand (37°48'S 175°17'E) in soil modified with additional organic material at pH ~4.3. The site has a mean annual rainfall of about 1200 mm with moderate temperatures (min./max. 0°C/29°C).

Table 1

Harvest dates used to compare genotypic and seasonal variation of blueberry fruit of the fourteen cultivars. Fruit were harvested at the 50%-ripe stage, i.e., when 50% of the fruit on the plant were blue. Within each *Vaccinium* species, cultivars are listed in order of ripening from early to late (mean ripening date across three years)

| Cultivar | Species | Blueberry type | Year of release | 2009-2010 | 2010-2011 | 2011-2012 |
|-----------------|-----------------------------|----------------|-----------------|------------------|------------------|------------------|
| 'Blue Bayou' | <i>Vaccinium corymbosum</i> | HB | 2008 | 2 December 2009 | 1 December 2010 | 1 December 2011 |
| 'Sunset Blue' | <i>V. corymbosum</i> | HB | 2008 | 2 December 2009 | 7 December 2010 | 1 December 2011 |
| 'Blue Moon' | <i>V. corymbosum</i> | HB | 2008 | 10 December 2009 | 7 December 2010 | 13 December 2011 |
| 'Nui' | <i>V. corymbosum</i> | HB | 1989 | 15 December 2009 | 7 December 2010 | 19 December 2011 |
| 'Reka' | <i>V. corymbosum</i> | HB | 1989 | 15 December 2009 | 7 December 2010 | 22 December 2011 |
| 'Cosmopolitan' | <i>V. corymbosum</i> | HB | 2012 | 22 December 2009 | 21 December 2010 | 4 January 2012 |
| 'Brigitta Blue' | <i>V. corymbosum</i> | HB | 1977 | 6 January 2010 | 5 January 2011 | 2 January 2012 |
| 'Sky Blue' | <i>V. virgatum</i> | RE | 2008 | 31 January 2010 | 26 January 2011 | 3 February 2012 |
| 'Dolce Blue' | <i>V. virgatum</i> | RE | 2012 | 5 February 2010 | 22 January 2011 | 31 January 2012 |
| 'Centurion' | <i>V. virgatum</i> | RE | 1978 | 8 February 2010 | 26 January 2011 | 1 February 2012 |
| 'Rahi' | <i>V. virgatum</i> | RE | 1992 | 15 February 2010 | 27 January 2011 | 5 February 2012 |
| 'Ocean Blue' | <i>V. virgatum</i> | RE | 2008 | 16 February 2010 | 27 January 2011 | 5 February 2012 |
| 'Velluto Blue' | <i>V. virgatum</i> | RE | 2012 | 14 February 2010 | 11 February 2011 | 21 February 2012 |
| 'Centra Blue' | <i>V. virgatum</i> | RE | 2006 | 22 February 2010 | 1 March 2011 | 8 March 2012 |

HB = highbush; RE = rabbiteye.

The field trial was established as a randomised complete block design with four replications of five plants of each cultivar except for the cultivars 'Brigitta Blue', 'Nui', 'Centurion' and 'Rahi' which had a single replication.

The blueberry plants under evaluation were planted in the ground in winter 2007 (June–July) and berries were harvested for three consecutive years (summers 2009–2010, 2010–2011 and 2011–2012). In this work, summer seasons are indicated as follows: 2009–2010 = 2010, 2010–2011 = 2011 and 2011–2012 = 2012.

For each cultivar, 1Kg of fruit was collected per plot when about 50% of the fruit were estimated to be ripe (fully blue). For each cultivar and year of evaluation the mean ripening dates are reported in Table 1.

Fruit weight, firmness and the fruit diameter were assessed on a sub-sample of 20 uniform fully ripe fruit. The assessments were done within 2 h from harvest. For the soluble solids content and titratable acidity assessments a juice sample was extracted from a sub-sample of 100 g of uniform fruit. For the polyphenol and antioxidant assessments, a sub-sample of 100 g of uniform fruit was stored at -20°C until it was analysed.

2.3. HPLC analysis of blueberry anthocyanins

Sample preparation, HPLC analysis and confirmation of identity of compounds by mass spectroscopy were carried out as described previously [29].

2.4. Ferric reducing antioxidant power (FRAP) assay

The measurements were carried out as described previously [30]. The final results were corrected for dilution, and expressed as $\mu\text{Mol Trolox equivalents}/100\text{ g}$ fresh material. All determinations were performed in triplicate.

2.5. Fruit firmness

Fruit firmness was assessed with FirmTech 2 (BioWorks, Inc.) and the results were expressed as force required (g) to generate a 1-mm deflection on the fruit surface. Fruit was placed on the FirmTech 2 plate on its side so that it would be compressed equatorially, and the fruit diameter was also recorded at the same time as the firmness.

2.6. Soluble solid content (SSC) and Titratable acidity (TA)

Soluble solid content (SSC) and titratable acidity (TA) were determined using freshly prepared juice. For each replication of each cultivar, 100 g of sound fruit was pureed using a hand-held blender, the resulting pulp was strained through two layers of cheesecloth and the juice was collected. The SSC was measured using a digital refractometer (model PR-101, Atago Co., Tokyo, Japan); TA (expressed as citric acid) was measured by titrating two 100-mL samples of 5% extracted juice with 0.1 mol L⁻¹ NaOH to pH 8.1.

2.7. Statistical analysis

Data were analysed using analysis of variance, testing effects of species against variation between cultivars within species; cultivars, year and species by year interaction against the cultivar by year interaction; and the cultivar by year interaction against the rep to rep variation within cultivars and years. Residuals were inspected to check the assumptions of analysis of variance; in a number of cases data were log-transformed to stabilise the variance. Means presented are on the original scale. Where data were log-transformed, the least significant differences (LSDs) on the log-scale were back-transformed to give least significant ratios (LSR); two means were significantly different if the larger was more than LSR times the smaller. Since many effects were significant, the percentage variation accounted for by each effect was calculated (effect sum of squares/total sum of squares). This helped indicate where the largest differences were for each measure. Correlations between the measures were calculated using the means for each cultivar in each year, and a principal component biplot produced (based on the correlation matrix). The analysis was done with GenStat (version 16, 2013, VSNi Ltd, Hemel Hempstead, UK).

3. Results and discussion

3.1. Polyphenolic compounds

The total ACY content varied significantly between the two *Vaccinium* species and amongst cultivars within species (Table 2A). According to our results the majority of the variation (82%) derived from the genetics, with the species accounting for 59% of the total variation and the cultivar within the species for a further 23%. The remaining variation was reported to be a consequence of interactions between species and year and between cultivar and year while the year effect on its own was non-significant. The total ACY of *V. corymbosum* fruit was significantly lower than those of *V. virgatum* with the mean value of the three years of assessment of 138 and 265 mg/100 g respectively. The ACY yearly mean values for *V. corymbosum* were also consistently lower than those of *V. virgatum* (Table 3).

Seasonal variations were relatively minor for *V. corymbosum* but *V. virgatum* fruit all exhibited a high value in 2012, especially 'Dolce Blue' and 'Centurion'. 'Centurion' also had the highest mean anthocyanin content over the three years (356 mg/100 g) (Table 3) and the highest anthocyanin content over all cultivars in 2010 and 2012, but not in 2011. 'Dolce Blue' came a close second for total ACY content and the rest of the *V. virgatum* fruit were similar (around 240 mg/100 g). 'Cosmopolitan' and 'Sunset Blue' fruit had the lowest mean anthocyanin content (around 100 mg/100 g). Although there were some variations in the pattern of anthocyanin content variation from year to year (Table 3), they were smaller than the general trends for cultivar. The variation between years was not significant and overall accounted only for 1% of the total variation (Table 2A). Similar results were also reported previously [29].

The cultivars appear to have responded differently to weather variations within each season. The fruit from the majority of *V. corymbosum* cultivars listed in Table 1 were harvested in December and had markedly lower anthocyanin contents than the late cultivars, which were harvested in January–March. Examination of weather records for the three growing seasons (not shown) detected no significant differences in either temperature or solar radiation intensity between December and January–March of any year. There was, however, lower temperature and solar intensity all through the 2012 season, which had no significant effect on the anthocyanin contents of the fruit for the cultivars considered in this study (Table 3). If anything, this parameter was slightly higher in 2012 for *V. virgatum* fruit in general but markedly so for 'Dolce Blue' and 'Centurion'. It therefore appears that the observed differences are related to genetic differences between the early ripening *V. corymbosum* cultivars and the later ripening *V. virgatum*

Table 2
The analysis of variance results for the polyphenol components of blueberries and fruit traits

| Table 2A – ANOVA | | ACY(y) | | TPH(y) | | FRAP(y) | | TM(y) | | AA | | CA(y) | |
|-------------------------------|------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
| Source of variation | d.f. | p-value | % variation | p-value | % variation | p-value | % variation | p-value | % variation | p-value | % variation | p-value | % variation |
| Species (S)* | 1 | <.001 | 59% | <.001 | 60% | <.001 | 33% | 0.001 | 49% | 0.005 | 36% | <.001 | 25% |
| Cultivar within Species** (C) | 12 | <.001 | 23% | <.001 | 17% | 0.001 | 12% | <.001 | 34% | <.001 | 37% | 0.061 | 6% |
| Year (Y) ** | 2 | 0.245 | 1% | <.001 | 11% | <.001 | 42% | 0.005 | 3% | 0.004 | 6% | <.001 | 46% |
| S x Y** | 2 | <.001 | 5% | 0.221 | 1% | 0.005 | 3% | 0.006 | 3% | 0.001 | 8% | <.001 | 14% |
| C x Y*** | 24 | 0.001 | 6% | 0.003 | 6% | 0.004 | 5% | 0.003 | 6% | <.001 | 10% | <.001 | 6% |
| Plot to plot | 62 | | 6% | | 6% | | 5% | | 6% | | 5% | | 4% |

| Table 2B – ANOVA | | Q(y) | | FW (y) | | FD | | F | | SSC | | TA | |
|-------------------------------|------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
| Source of variation | d.f. | p-value | % variation | p-value | % variation | p-value | % variation | p-value | % variation | p-value | % variation | p-value | % variation |
| Species (S)* | 1 | 0.015 | 4% | 0.985 | 0% | 0.594 | 2% | 0.869 | 0% | 0.029 | 23% | <.001 | 57% |
| Cultivar within Species** (C) | 12 | 0.016 | 6% | <.001 | 87% | <.001 | 78% | <.001 | 67% | <.001 | 46% | <.001 | 32% |
| Year (Y) ** | 2 | <.001 | 84% | 0.059 | 2% | 0.218 | 1% | 0.004 | 7% | 0.043 | 5% | 0.002 | 5% |
| S x Y** | 2 | 0.869 | 0% | 0.880 | 0% | 0.721 | 0% | 0.289 | 1% | 0.033 | 5% | 0.864 | 0% |
| C x Y*** | 24 | <.001 | 4% | 0.001 | 6% | <.001 | 10% | 0.017 | 11% | <.001 | 15% | <.001 | 7% |
| Plot to plot | 62 | | 2% | | 6% | | 9% | | 14% | | 6% | | 4% |

*Tested against Cultivar within Species (12 df). **Tested against Cultivar within Species.Year (24 df). ***Tested against plot to plot variation within species - cultivar - year (62 df). (y) log transformed. ACY = total anthocyanin content; TPH = total phenolic content; FRAP = Ferric reducing antioxidant power; TM = total malvidin; AA = acylated anthocyanins; CA = chlorogenic acid; Q = quercetin glycosides; FW = fruit weight; FD = fruit diameter; F = fruit firmness; SSC = soluble solid content; TA = titratable acidity.

cultivars. Blueberry cultivars with high anthocyanin contents can have an advantage in marketing to consumers because anthocyanins have been reported to have health benefits, as previously discussed. This study agrees with previous reports [5, 23, 24, 28, 31–33] in finding considerable variation in fruit anthocyanin contents between cultivars (Table 3).

3.2. Total Polyphenol Content (TPH)

Similarly to ACY, the TPH varied significantly between the two *Vaccinium* species and between cultivars with the genetics accounting for 77% of the total variation (Table 2A). Significant differences were also reported between years for 11% of the total variance, while the remaining fraction resulted from interactions between year and cultivar. The TPH values of *V. corymbosum* fruit were significantly lower than those of *V. virgatum* with the mean value of the three years of assessment of 178 and 340 mg/100 g respectively. The TPH yearly mean values for *V. corymbosum* were also consistently lower than those of *V. virgatum* (Table 3). The between-cultivar variation in this parameter (Table 3) was similar in profile to that of total ACY, but generally less marked. The cultivars with the highest mean TPH over the three years of evaluation were ‘Centurion’ and ‘Dolce Blue, followed by ‘Centra Blue’ (418, 395 and 382 mg/100 g respectively) (Table 3). ‘Brigitta Blue’ fruit had the lowest mean TPH (138 mg/100 g). Significant differences were found from year to year, i.e. TPH mean values of ‘Ocean Blue’ fruit in 2012 were lower than those of years 2010 and 2011 (Table 3), however they were smaller than the genetic differences.

Relatively few cultivars showed large seasonal variations in TPH. When it did occur, however, it was inverse to that of total ACY, with marked decreases in the rabbiteye cultivars ‘Dolce Blue’ and ‘Centurion’ in the 2012 season. These cultivars had exceptionally high total ACY in 2012. Apparently, cultivars that respond to cooler weather during ripening by increasing ACY production do so at the expense of other polyphenols.

Table 3

Genotypic and seasonal variation of total anthocyanin content (ACY), total phenolic content (TPH) and Ferric reducing antioxidant power (FRAP) of blueberries with means for each cultivar, year and specie. Within each *Vaccinium* species cultivars are listed in order of ripening from early to late (mean ripening date across three years)

| Cultivars | ACY (mg/ 100 g) | | | | TPH (mg/100 g) | | | | FRAP (μ mol Trolox eq/100 g) | | | |
|-----------------------------|-----------------|------|---------------------|--------------|-----------------|------|---------------------|--------------|-----------------------------------|------|---------------------|----------------|
| | 2010 | 2011 | 2012 | mean | 2010 | 2011 | 2012 | mean | 2010 | 2011 | 2012 | mean |
| 'Blue Bayou' | 172 | 183 | 158 | 171 | 243 | 251 | 179 | 224 | 1804 | 3049 | 1149 | 2001 |
| 'Sunset Blue' | 111 | 111 | 85 | 102 | 169 | 178 | 124 | 157 | 1269 | 2192 | 721 | 1394 |
| 'Blue Moon' | 147 | 191 | 158 | 165 | 209 | 236 | 147 | 197 | 1673 | 2880 | 959 | 1837 |
| 'Nui' | 161 | 201 | 189 | 184 | 213 | 263 | 189 | 222 | 1462 | 3143 | 1333 | 1979 |
| 'Reka' | 76 | 128 | 160 | 122 | 141 | 185 | 147 | 157 | 1093 | 2365 | 941 | 1466 |
| 'Cosmopolitan' | 85 | 124 | 85 | 98 | 146 | 168 | 146 | 153 | 1173 | 1859 | 969 | 1334 |
| 'Brigitta Blue' | 120 | 114 | 142 | 125 | 140 | 142 | 132 | 138 | 1073 | 1597 | 994 | 1221 |
| Mean <i>V. corymbosum</i> | 125 | 150 | 140 | 138 \pm 35 | 180 | 203 | 152 | 178 \pm 35 | 1364 | 2441 | 1009 | 1605 \pm 325 |
| 'Sky Blue' | 219 | 187 | 241 | 216 | 300 | 301 | 224 | 275 | 2137 | 3045 | 1618 | 2266 |
| 'Dolce Blue' | 322 | 234 | 387 | 314 | 438 | 360 | 387 | 395 | 3117 | 3573 | 2578 | 3089 |
| 'Centurion' | 327 | 238 | 503 | 356 | 466 | 365 | 425 | 418 | 3701 | 3792 | 3046 | 3513 |
| 'Rahi' | 250 | 206 | 203 | 220 | 301 | 274 | 275 | 283 | 2069 | 2852 | 1937 | 2286 |
| 'Ocean Blue' | 205 | 232 | 263 | 233 | 315 | 344 | 182 | 280 | 2185 | 3681 | 1102 | 2323 |
| 'Velluto Blue' | 243 | 265 | 278 | 260 | 391 | 398 | 216 | 346 | 2904 | 3904 | 1526 | 2892 |
| 'Centra Blue' | 261 | 215 | 288 | 255 | 419 | 378 | 348 | 382 | 2912 | 4007 | 2610 | 3176 |
| Mean <i>V. virgatum</i> | 261 | 225 | 309 | 265 \pm 52 | 376 | 346 | 294 | 340 \pm 60 | 2718 | 3551 | 2060 | 2792 \pm 503 |
| LSR | Individual year | | Mean of three years | | Individual year | | Mean of three years | | Individual year | | Mean of three years | |
| Species (S) to S | 130% | | 128% | | 125% | | 122% | | 127% | | 123% | |
| Cultivar (C) to C | 144% | | 117% | | 140% | | 114% | | 146% | | 115% | |
| Year (Y) to Y (for S means) | 116% | | | | 115% | | | | 117% | | | |
| Y to Y (for C means) | 133% | | | | 133% | | | | 139% | | | |

ACY = total anthocyanin content; TPH = total phenolic content; FRAP = Ferric reducing antioxidant power. In this work, summer seasons are indicated as follows: 2009-2010 = 2010, 2010-2011 = 2011 and 2011-2012 = 2012. LSR = Least significant ratios. If the least significant ratio is 130% then when comparing two means, if the larger is more than 130% of the smaller (i.e. 30% higher) they are significantly different. In this table for Species 130% relates to the means for individual years and the 128% relates to the Mean column (i.e. the mean across the three years).

3.3. FRAP

FRAP, a measure of antioxidant capacity, correlates strongly with TPH and ACY, but there are clearly contributions from other compounds. The overall difference between *V. corymbosum* and *V. virgatum* species was significant and accounted for 33% of the total variation while the differences between cultivars are relatively small and accounted for 12% of the total variation (Table 2A). Similar results have been found previously [7]: their assessment of antioxidant activity for *Vaccinium* species was genotype related, with *V. virgatum* higher than *V. corymbosum*.

In our work, however, the majority of the variation for FRAP was attributable to the differences between years (42%). All cultivars exhibited approximately mean values in 2010, but well above mean in 2011 and below mean in 2012 (Table 3). Nothing in the weather records, or any other data collected, appears to explain the high FRAP values in 2011.

Amongst all the cultivars, 'Centurion' had the highest FRAP mean value while 'Brigitta Blue' had the lowest (Table 3).

3.4. Polyphenol components

Compositional analysis of the polyphenols revealed that malvidin (as the sum of the three glycosidic forms) was consistently the major anthocyanin component, comprising 30–50% of the mean total anthocyanin content (data not

shown). Total Malvidins (TM) showed a very similar between-species profile to total ACY and little seasonal variation, which accounted for only 3% of the total variance (Table 4). TM ranged between 30 mg/100 g ('Cosmopolitan') to 147 mg/100 g ('Dolce Blue') with *V. virgatum* fruit having two-fold higher content than *V. corymbosum*. Acylated anthocyanins (AA) were minor components among the polyphenols and showed a generally inverse relationship with total ACY. The AA content of *V. corymbosum* fruit was generally higher than that of *V. virgatum*; significant differences were found between cultivars (Table 2A) with 'Blue Moon' being the highest at 41 mg/100 g (Table 4). Seasonal variation of AA was minor in *V. corymbosum* fruit, but major in *V. virgatum*, with 'Rahi' and 'Velluto Blue' producing almost none in 2012, whereas the other cultivars had greatly elevated levels compared with the other seasons.

Chlorogenic acid (CA, Table 4) is the main non-anthocyanin polyphenol in blueberries. This parameter was generally higher in *V. virgatum* with 'Velluto Blue' the highest at 85 mg/100 g and all *V. corymbosum* between 20 and 40 mg/100 g. Seasonal variation was marked and completely different from ACY and TPH, with the highest content in 2010 and much less in 2011 and 2012. Quercetin (Q, Table 4), showed little between-cultivar variation with the exception of 'Blue Bayou', which stood out at 52 mg/100 g. Seasonal variation was marked (84% of the total variation – Table 2A) and completely opposite to CA, with very little in any fruit from 2010 but much more in all fruit in 2011 and 2012. CA and Q both show marked seasonal variations that are not obviously explained by weather records. Their variation may be linked to weather during a short and as yet unidentified period in the growing season, rather than the average conditions.

3.5. Quality traits

Blueberry cultivars with large fruit are particularly advantageous to the majority of New Zealand growers who hand-harvest their crop. Fruit weight (FW) varied significantly with cultivar which accounted for the majority of the total variation (87%). The individual FW of cultivars within the species varied greatly (Table 5). Within *V. corymbosum* we found the cultivar with the highest and lowest individual FW. 'Nui' had the highest mean FW and no significant variation was found between years (Table 5). The cultivar with the lowest average FW was 'Blue Bayou', and its fruit size was consistently small across the years.

The anthocyanins are found only in the skin of blueberries, so it would be expected that small fruit, with relatively more skin area, would tend towards high total ACY. This relationship holds for 'Dolce Blue' and 'Centurion', but not for small 'Blue Bayou' fruit or large 'Blue Moon', 'Nui' or 'Cosmopolitan'. This suggests that small fruit size is one of a number of influences on total ACY and has been reported previously [4, 29].

Fruit diameter (FD) reflected greatly the FW for species and cultivar effects in our study (Tables 2B and 5). The difference in FD among cultivars can be of practical importance to New Zealand growers because the fruit can be packed and sold by diameter with a premium price for those fruit with diameter over 18 mm.

Blueberry ripening is accompanied by changes of the fruit firmness, decreases in acidity and increases in sugars [34]; the fruit firmness varies with the stage of maturity [35]. Once initial ripeness has been achieved a further process of override softening occurs which is accompanied by further decrease of acidity and increase in sugars. Consequently we felt the need to develop a standard sampling procedure which allowed us to identify when 50% of the fruit on the bush was ripe and before any sign of softening occurred. The regular fruit harvest at 50% of maturity has proven to be a reliable method and of critical importance when comparing results of a number of different cultivars over a number of different seasons.

Fruit of *V. virgatum* has been reported to be firmer than that of *V. corymbosum* [36–39]. According to our results the difference between species was minor and the majority of the variation was found between cultivars (67% of the total variation – Table 2B). Firmness varied greatly among cultivars (Table 5), the firmest fruit was harvested from 'Centra Blue' (235 g/mm) and the softest were from 'Reka' (159 g/mm) and 'Dolce Blue' (160 g/mm) (Table 5). Previously authors reported a similar average firmness for 'Reka' (154 g/mm) and defined a firmness of 150–160 g/mm as above the average across their germplasm collection [37]. 'Reka' was released by PFR in 1989 and since then a lot of effort was put into repeating cycles of selection for firmer fruit, resulting in cultivars such as 'Centra Blue', 'Blue Bayou' and 'Velluto Blue' that have improved firmness. There was no significant seasonal variation in firmness (Table 5).

V. corymbosum fruit had generally lower soluble solids content (SSC) and higher total acidity (TA) than *V. virgatum* (Table 6). The differences in SSC and TA between cultivars were significant. SSC ranged between 9.1 to 14.9% with

Table 4

Genotypic and seasonal variation of polyphenol components (mg/100 g) of blueberries, with means for each cultivar, year and specie. Within each *Vaccinium* species cultivars are listed in order of ripening from early to late (mean ripening date across three years)

| Cultivars | TM | | | AA | | | CA | | | Q | | | | | | |
|-----------------------------|-----------------|-----------------|-----------------|---------------------|-----------------|-----------------|-----------------|---------------------|-----------------|-----------------|-----------------|---------------------|-----------------|-----------------|-----------------|---------------------|
| | 2010 | 2011 | 2012 | Mean | 2010 | 2011 | 2012 | Mean | 2010 | 2011 | 2012 | Mean | | | | |
| | Individual year | Individual year | Individual year | Mean of three years | Individual year | Individual year | Individual year | Mean of three years | Individual year | Individual year | Individual year | Mean of three years | | | | |
| 'Blue Bayou' | 71 | 76 | 68 | 72 | 9 | 30 | 22 | 20 | 38 | 26 | 3 | 22 | 13 | 82 | 62 | 52 |
| 'Sunset Blue' | 40 | 42 | 33 | 39 | 9 | 19 | 10 | 13 | 62 | 32 | 2 | 32 | 9 | 39 | 38 | 29 |
| 'Blue Moon' | 56 | 68 | 54 | 59 | 37 | 49 | 36 | 41 | 47 | 20 | 1 | 23 | 9 | 61 | 46 | 38 |
| 'Nui' | 78 | 80 | 67 | 75 | 19 | 28 | 27 | 25 | 57 | 36 | 10 | 34 | 5 | 46 | 49 | 33 |
| 'Reka' | 30 | 46 | 57 | 44 | 9 | 30 | 29 | 23 | 57 | 30 | 3 | 30 | 9 | 37 | 38 | 28 |
| 'Cosmopolitan' | 23 | 42 | 25 | 30 | 26 | 27 | 20 | 25 | 44 | 18 | 1 | 21 | 6 | 42 | 20 | 23 |
| 'Brigitta Blue' | 45 | 63 | 51 | 53 | - | 1 | 14 | 7 | 39 | 17 | 19 | 25 | 7 | 28 | 33 | 23 |
| Mean <i>V. corymbosum</i> | 49 | 60 | 51 | 53 ± 17 | 18 | 26 | 23 | 22 ± 11 | 49 | 26 | 5.6 | 27 ± 5 | 8.3 | 48 | 41 | 32 ± 10 |
| 'Sky Blue' | 72 | 79 | 92 | 81 | 5 | 6 | 18 | 10 | 70 | 29 | 16 | 38 | 7 | 46 | 30 | 28 |
| 'Dolce Blue' | 135 | 126 | 180 | 147 | 4 | 1 | 21 | 9 | 115 | 38 | 28 | 61 | 5 | 38 | 36 | 26 |
| 'Centurion' | 91 | 110 | 146 | 116 | 6 | 0 | 23 | 10 | 91 | 31 | 37 | 53 | 11 | 25 | 56 | 31 |
| 'Rahi' | 112 | 110 | 86 | 102 | 6 | 2 | 0 | 3 | 60 | 33 | 43 | 45 | 3 | 36 | 23 | 21 |
| 'Ocean Blue' | 73 | 98 | 111 | 94 | 5 | 5 | 19 | 10 | 103 | 44 | 26 | 58 | 6 | 33 | 25 | 21 |
| 'Velluto Blue' | 90 | 153 | 121 | 121 | 4 | 7 | 0 | 4 | 140 | 58 | 49 | 85 | 8 | 38 | 30 | 25 |
| 'Centra Blue' | 59 | 73 | 100 | 77 | 3 | 1 | 17 | 7 | 126 | 36 | 32 | 65 | 9 | 28 | 29 | 22 |
| Mean <i>V. virgatum</i> | 90 | 107 | 119 | 105 ± 25 | 4.7 | 3.1 | 14 | 7.6 ± 3 | 101 | 38 | 33 | 58 ± 15 | 7.0 | 35 | 33 | 25 ± 4 |
| LSR | Individual year | Individual year | Individual year | Mean of three years | Individual year | Individual year | Individual year | Mean of three years | Individual year | Individual year | Individual year | Mean of three years | Individual year | Individual year | Individual year | Mean of three years |
| Species (S) to S | | 143% | | 140% | | 11 | | 10 | 165% | | | 147% | | 137% | | 129% |
| Cultivar (C) to C | | 158% | | 123% | | 13 | | 6 | 229% | | | 134% | | 147% | | 120% |
| Year (Y) to Y (for S means) | | 117% | | | | 6 | | | 155% | | | | | 129% | | |
| Y to Y (for C means) | | 139% | | | | 8 | | | 215% | | | | | 136% | | |

TM = total malvidin; AA = acylated anthocyanins; CA = chlorogenic acid; Q = quercetin glycosides. In this work, summer seasons are indicated as follows: 2009-2010 = 2010, 2010-2011 = 2011 and 2011-2012 = 2012.

Table 5

Genotypic and seasonal variation of fruit weight (FW), fruit diameter (FD) and firmness (F). Within each *Vaccinium* species cultivars are listed in order of ripening from early to late (mean ripening date across three years)

| Cultivars | FW (g) | | | | FD (mm) | | | | F (g/mm) | | | |
|-----------------------------|-----------------|------|------|---------------------|-----------------|------|------|---------------------|-----------------|------|------|---------------------|
| | 2010 | 2011 | 2012 | Mean | 2010 | 2011 | 2012 | Mean | 2010 | 2011 | 2012 | Mean |
| 'Blue Bayou' | 1.2 | 1.1 | 1.2 | 1.2 | 14.2 | 13.9 | 13.9 | 14.0 | 233 | 215 | 225 | 224 |
| 'Sunset Blue' | 2.5 | 2.2 | 2.3 | 2.4 | 18.7 | 18.0 | 19.1 | 18.6 | 215 | 183 | 177 | 189 |
| 'Blue Moon' | 3.2 | 3.1 | 3.0 | 3.1 | 20.6 | 20.5 | 20.4 | 20.5 | 218 | 201 | 216 | 212 |
| 'Nui' | 3.3 | 3.1 | 3.1 | 3.2 | 21.3 | 21.4 | 22.1 | 21.6 | 179 | 161 | 171 | 171 |
| 'Reka' | 1.9 | 2.0 | 2.3 | 2.1 | 17.4 | 18.7 | 19.0 | 18.4 | 140 | 166 | 172 | 159 |
| 'Cosmopolitan' | 2.8 | 2.8 | 3.7 | 3.1 | 19.5 | 20.2 | 22.0 | 20.6 | 202 | 162 | 159 | 174 |
| 'Brigitta Blue' | 2.4 | 2.6 | 3.8 | 2.9 | 18.5 | 14.6 | 22.8 | 18.6 | 164 | 168 | 176 | 170 |
| Mean <i>V. corymbosum</i> | 2.5 | 2.4 | 2.8 | 2.6 ± 0.7 | 19 | 18 | 20 | 19 ± 2.5 | 193 | 179 | 185 | 186 ± 24 |
| 'Sky Blue' | 2.9 | 2.8 | 2.7 | 2.8 | 19.1 | 19.3 | 19.5 | 19.3 | 206 | 212 | 191 | 203 |
| 'Dolce Blue' | 1.6 | 1.7 | 1.7 | 1.7 | 16.3 | 15.6 | 16.3 | 16.1 | 164 | 162 | 154 | 160 |
| 'Centurion' | 1.6 | 2.0 | 2.1 | 1.9 | 16.4 | 17.3 | 13.1 | 15.6 | 219 | 205 | 200 | 208 |
| 'Rahi' | 2.4 | 2.5 | 2.5 | 2.5 | 18.3 | 18.3 | 18.5 | 18.4 | 181 | 198 | 215 | 198 |
| 'Ocean Blue' | 1.8 | 2.1 | 2.2 | 2.0 | 16.2 | 16.7 | 17.8 | 16.9 | 193 | 209 | 203 | 202 |
| 'Velluto Blue' | 2.8 | 2.1 | 2.4 | 2.4 | 18.6 | 17.7 | 18.5 | 18.2 | 222 | 198 | 199 | 208 |
| 'Centra Blue' | 2.0 | 1.9 | 2.7 | 2.2 | 16.2 | 17.4 | 18.1 | 17.3 | 264 | 229 | 211 | 235 |
| Mean <i>V. virgatum</i> | 2.2 | 2.2 | 2.3 | 2.2 ± 0.4 | 17 | 17 | 17 | 17 ± 1 | 207 | 202 | 196 | 202 ± 22 |
| LSR | Individual year | | | Mean of three years | Individual year | | | Mean of three years | Individual year | | | Mean of three years |
| Species (S) to S | 146% | | | 145% | 2.6 | | | 2.5 | 31 | | | 29 |
| Cultivar (C) to C | 152% | | | 124% | 3.0 | | | 1.5 | 41 | | | 18 |
| Year (Y) to Y (for S means) | 112% | | | | 1.1 | | | | 14 | | | |
| Y to Y (for C means) | 124% | | | | 1.9 | | | | 32 | | | |

FW = fruit weight; FD = fruit diameter; F = fruit firmness. In this work, summer seasons are indicated as follows: 2009-2010 = 2010, 2010-2011 = 2011 and 2011-2012 = 2012.

'Cosmopolitan' fruit having the lowest percentage of soluble solids and fruit of cultivars 'Rahi' and 'Ocean Blue' having the highest mean SSC. 'Cosmopolitan' and 'Rahi' also had the lowest and highest TA content respectively. There were differences in the SSC/TA ratio among cultivars (Table 6), mainly arising from differences in TA. 'Cosmopolitan' and 'Rahi' had the lowest and highest ratios in absolute terms (9.4 and 89.5 respectively). There was a significant ~3-fold difference between the SSC/TA mean ratio of *V. corymbosum* (18) and *V. virgatum* (53). Sugar and organic acids have an important influence on the sensory quality of fruit. A good flavoured blueberry should have high sugar and high acidity. Although not all blueberries with high SSC will necessarily be of good taste, a low SSC makes good taste unlikely. In a previous report, Beaudry [40] suggested that blueberry fruit should contain >10% SSC, 0.3–1.3% TA and a SSC/TA ratio between 10 and 33. Based on these quality indications, all cultivars evaluated in this report have acceptable SSC content except for 'Cosmopolitan', lower TA was found in 'Sky Blue', 'Dolce Blue', 'Rahi' and 'Ocean Blue', and unbalanced SSC/TA ratios were found in 'Cosmopolitan', 'Sky Blue', 'Dolce Blue', 'Rahi', 'Ocean Blue' and 'Velluto Blue'. Other researchers have found no correlation between SSC and sensory scores for intensity of sweetness [39], therefore further investigation is needed to determine whether there is a relationship between SSC and sweetness in blueberries.

3.6. Differences in phenolic components and fruit traits between the *Vaccinium* species and during the protracted harvest season

V. corymbosum cultivars share many characteristics, as do *V. virgatum* cultivars, but the two species show distinct differences. The former is early ripening (Table 1), lower in anthocyanins and other polyphenols, and has very variable

Table 6

Genotypic and seasonal variation of soluble solid content (SSC), titratable acidity (TA) and their ratio SSC/TA. Within each *Vaccinium* species cultivars are listed in order of ripening from early to late (mean ripening date across three years)

| Cultivars | SSC (%) | | | | TA (%) | | | | SSC/TA ratio | | | |
|-----------------------------|-----------------|------|------|---------------------|-----------------|------|------|---------------------|-----------------|------|------|---------------------|
| | 2010 | 2011 | 2012 | Mean | 2010 | 2011 | 2012 | Mean | 2010 | 2011 | 2012 | Mean |
| 'Blue Bayou' | 15.0 | 13.4 | 12.9 | 13.8 | 0.57 | 0.62 | 0.43 | 0.54 | 26.2 | 21.5 | 29.7 | 25.8 |
| 'Sunset Blue' | 13.1 | 9.7 | 10.7 | 11.2 | 0.64 | 0.53 | 0.46 | 0.54 | 20.4 | 18.3 | 23.5 | 20.7 |
| 'Blue Moon' | 13.2 | 12.7 | 12.1 | 12.7 | 0.68 | 0.57 | 0.62 | 0.62 | 19.3 | 22.4 | 19.5 | 20.4 |
| 'Nui' | 11.9 | 12.1 | 9.8 | 11.2 | 0.86 | 0.73 | 0.82 | 0.80 | 13.8 | 16.5 | 12.0 | 14.1 |
| 'Reka' | 10.1 | 10.2 | 10.6 | 10.3 | 0.57 | 0.53 | 0.42 | 0.51 | 17.5 | 19.2 | 25.4 | 20.7 |
| 'Cosmopolitan' | 9.8 | 8.6 | 9.0 | 9.1 | 1.10 | 0.81 | 1.04 | 0.98 | 8.9 | 10.6 | 8.6 | 9.4 |
| 'Brigitta Blue' | 11.6 | 13.6 | 10.9 | 12.0 | 0.75 | 0.78 | 0.70 | 0.74 | 15.4 | 17.4 | 15.6 | 16.2 |
| Mean <i>V. corymbosum</i> | 12 | 11 | 11 | 11 ± 1.5 | 0.74 | 0.65 | 0.64 | 0.68 ± 0.2 | 17 | 18 | 19 | 18 ± 5.4 |
| 'Sky Blue' | 14.1 | 14.0 | 13.3 | 13.8 | 0.35 | 0.22 | 0.24 | 0.27 | 40.8 | 63.8 | 54.2 | 52.9 |
| 'Dolce Blue' | 13.7 | 14.0 | 13.4 | 13.7 | 0.26 | 0.30 | 0.22 | 0.26 | 52.2 | 45.9 | 60.9 | 53.0 |
| 'Centurion' | 12.7 | 15.1 | 14.1 | 14.0 | 0.32 | 0.58 | 0.45 | 0.45 | 39.3 | 25.8 | 31.2 | 32.1 |
| 'Rahi' | 14.9 | 14.8 | 14.9 | 14.9 | 0.16 | 0.19 | 0.16 | 0.17 | 95.6 | 77.9 | 95.0 | 89.5 |
| 'Ocean Blue' | 14.5 | 15.4 | 14.8 | 14.9 | 0.21 | 0.23 | 0.16 | 0.20 | 67.8 | 67.0 | 92.8 | 75.8 |
| 'Velluto Blue' | 13.8 | 12.2 | 12.9 | 13.0 | 0.40 | 0.30 | 0.30 | 0.33 | 34.6 | 40.6 | 43.5 | 39.6 |
| 'Centra Blue' | 12.1 | 12.9 | 13.5 | 12.9 | 0.59 | 0.48 | 0.36 | 0.47 | 20.7 | 27.0 | 37.8 | 28.5 |
| Mean <i>V. virgatum</i> | 14 | 14 | 14 | 14 ± 0.8 | 0.33 | 0.33 | 0.27 | 0.31 ± 0.1 | 50 | 50 | 59 | 53 ± 23 |
| LSD | Individual year | | | Mean of three years | Individual year | | | Mean of three years | Individual year | | | Mean of three years |
| Species (S) to S | 1.4 | | | 1.3 | 0.15 | | | 0.14 | 15.9 | | | 15.3 |
| Cultivar (C) to C | 1.6 | | | 0.8 | 0.17 | | | 0.08 | 17.5 | | | 9.2 |
| Year (Y) to Y (for S means) | 0.9 | | | | 0.07 | | | | 6.8 | | | |
| Y to Y (for C means) | 1.1 | | | | 0.11 | | | | 9.9 | | | |

SSC = soluble solid content; TA = titratable acidity. In this work, summer seasons are indicated as follows: 2009-2010 = 2010, 2010-2011 = 2011 and 2011-2012 = 2012.

fruit weight, size and SSC/TA values (Tables 3–6). The latter is late ripening, higher in anthocyanins and polyphenols, with consistently small fruit and SSC/TA values. Neither species is “better”, but they need to be targeted to different markets and applications. For example, *V. virgatum* cultivars may be more convincingly marketed as “high health” based on their high anthocyanin content, especially ‘Dolce Blue’ and ‘Centurion’. Often, fruit from *V. virgatum* cultivars are machine harvested because the small fruit size does not make hand harvesting economically viable (J.S personal observation). *V. virgatum* fruit also contain a higher accumulation of sclereids that make the fruit texture ‘seedy’ (gritty). Seediness is not discussed in this study, but has been reported by previous authors who have found it to be a genetic effect [41]. For some distributors the seediness of *V. virgatum* fruit might be a reason to reject the fruit lot and to opt for fruit of *V. corymbosum* which has less seediness.

It is perhaps inconvenient that growers cannot easily select an early- and late-maturing cultivar to extend the harvesting season, both with similar characteristics and equally applicable to the same market segment. Retail blueberries are not labelled by cultivar in New Zealand, so consumers may notice some inconsistency with fruit size and quality over the season. With such a prolonged harvest season it is therefore difficult to maintain a consistent fruit quality.

3.7. Correlations between phenolics and fruit quality traits

According to our results, the strongest phenotypic correlations were FW with FD ($r=0.88$), malvidin with total anthocyanins ($r=0.86$), and total phenolics with FRAP and with total anthocyanins ($r=0.85$ and 0.80 respectively,

Table 7

Matrix of phenotypic correlations between traits, based on mean values for each cultivar – year combination ($n=41$). Correlations >0.31 or <-0.31 are significant at $p=0.05$ (shown in bold and highlighted in grey)

| | TPH | ACY | AA | TM | CA | Q | FW | SSC | TA | F | FD |
|------|-------------|-------------|--------------|-------------|--------------|--------------|--------------|--------------|--------------|-------------|--------------|
| FRAP | 0.85 | 0.55 | -0.31 | 0.56 | 0.39 | 0.12 | -0.38 | 0.44 | -0.36 | 0.25 | -0.38 |
| TPH | - | 0.80 | -0.43 | 0.71 | 0.62 | -0.15 | -0.41 | 0.55 | -0.51 | 0.33 | -0.45 |
| ACY | | - | -0.19 | 0.86 | 0.33 | 0.06 | -0.32 | 0.54 | -0.55 | 0.19 | -0.43 |
| AA | | | - | -0.30 | -0.47 | 0.47 | 0.29 | -0.45 | 0.44 | -0.15 | 0.35 |
| TM | | | | - | 0.21 | 0.09 | -0.35 | 0.62 | -0.64 | 0.00 | -0.40 |
| CA | | | | | - | -0.63 | -0.22 | 0.30 | -0.25 | 0.31 | -0.22 |
| Q | | | | | | - | -0.12 | -0.12 | 0.01 | -0.06 | -0.12 |
| FW | | | | | | | - | -0.36 | 0.43 | -0.21 | 0.88 |
| SSC | | | | | | | | - | -0.66 | 0.45 | -0.50 |
| TA | | | | | | | | | - | -0.16 | 0.38 |
| F | | | | | | | | | | - | -0.28 |

ACY = total anthocyanin content; TPH = total phenolic content; FRAP = Ferric reducing antioxidant power; TM = total malvidin; AA = acylated anthocyanins; CA = chlorogenic acid; Q = quercetin glycosides; FW = fruit weight; FD = fruit diameter; F = fruit firmness; SSC = soluble solid content; TA = titratable acidity.

Table 7). Other authors have also found that the correlation between phenolic content and antioxidant activity is higher than that of anthocyanin and antioxidant capacity [5, 24].

A significant correlation was reported between antioxidant capacity and anthocyanin content ($r=0.61$) and antioxidant capacity with total phenolics ($r=0.89$) [24]. Similarly, Connor et al. [5], found that in blueberries, total phenolics correlated better with antioxidant capacity ($r=0.82$) than did total anthocyanin content ($r=0.73$). Anthocyanins and phenolics are secondary plant metabolites; they protect the plant against damaging photodynamic reactions by quenching the excited state of active oxygen species [42].

The correlations between the individual polyphenol compounds and total anthocyanin contents varied, showing that TM had the strongest positive correlation with ACY. The strongest negative correlations were found between SSC and TA ($r=-0.66$), malvidins and TA ($r=-0.64$) and CA and Q ($r=-0.63$). The selection work for specific characteristics of individual plants in a breeding population must take into consideration that negative correlations between traits might occur. Therefore different breeding strategies are required in order not to lose those fruit characteristics that are inversely correlated with what is selected for.

Evaluating a multitude of cultivars in test trials is common practice in breeding programmes and it is associated with considerable cost related to the cultivation and maintenance of large numbers of bushes and data collection from the trial. Amongst all the traits included in our work, determining the phytochemicals during the three years for all the cultivars was the most expensive part of the research. According to our results, all the traits analysed in this work except Q and AA from a single year collected from all the cultivars were highly correlated (correlations over 0.8) to the average across the three years (Table 8). Therefore, assessing these traits from a single year of data should be mostly reliable for individual cultivars.

In a previous study [43], the single-year results obtained for fruit weights and total anthocyanin contents were also highly correlated to the averages across the three years (Table 8), which makes evaluation of these traits from a single year and at an early stage of plant development reasonably achievable. It therefore appears that it would be practical to select promising crosses from a breeding programme in the third year of plant growth, with a low probability of missing high-performing plants that happened to perform unusually poorly in year 3. The correlation of the individual polyphenol compounds with the total anthocyanin contents in each year and over the three-year evaluation showed some significant differences (Table 7). Only TM showed consistently positive and significant correlations with ACY, each year and over the three-year period. CA had high positive correlations with ACY, but not in 2011. Overall, TM and CA were highly and positively correlated to the total anthocyanin content over the three years of evaluation. The correlations between AA and ACY, and Q and ACY, were negative over the entire evaluation, but not significantly so.

Table 8
Correlations between individual year results for blueberry cultivars and mean results across the three years for cultivars

| Individual year | ACY | TPH | FRAP | TM | AA | CA | Q | FW | FD | F | SSC | TA |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 2010 | 0.970 | 0.996 | 0.985 | 0.941 | 0.910 | 0.972 | 0.676 | 0.935 | 0.960 | 0.896 | 0.878 | 0.974 |
| 2011 | 0.881 | 0.943 | 0.912 | 0.940 | 0.947 | 0.884 | 0.872 | 0.967 | 0.841 | 0.953 | 0.926 | 0.955 |
| 2012 | 0.968 | 0.923 | 0.904 | 0.953 | 0.799 | 0.856 | 0.864 | 0.902 | 0.887 | 0.884 | 0.956 | 0.984 |

ACY = total anthocyanin content; TPH = total phenolic content; FRAP = Ferric reducing antioxidant power; TM = total malvidin; AA = acylated anthocyanins; CA = chlorogenic acid; Q = quercetin glycosides; FW = fruit weight; FD = fruit diameter; F = fruit firmness; SSC = soluble solid content; TA = titratable acidity.

4. Conclusions

This study showed that there was a wide variation in polyphenolic and quality traits between blueberry genotypes and species. We found that highbush (*V. corymbosum*) cultivars share many characteristics, as do rabbiteye (*V. virgatum*) cultivars, but the two species show distinct differences. The former is early ripening, lower in anthocyanins and other polyphenols, and has very variable fruit weight, size and SSC/TA values. The latter is late ripening, higher in anthocyanins and polyphenols, with consistently small fruit and SSC/TA values. These differences mean that cultivars from each species need to be targeted to different markets and applications. For example, *V. virgatum* cultivars could be marketed as “high health” based on their high anthocyanin content, especially ‘Dolce Blue’ and ‘Centurion’. Since growers cannot grow both an early- and a late-maturing cultivar, with similar characteristics to extend the harvesting season, consumers may notice some inconsistency with fruit size and quality over the season. This inconsistency could mean more acidic fruit in December, but sweeter later in the season, firm and soft fruit, large and small berries. The combination of cultivars in this study was designed to offer the widest possible harvest window; however, it gives high variation in fruit quality.

Even though it was not the primary objective of this paper, the information collected may also help to identify sources of breeding material for improving traits (e.g., increasing polyphenol content, or fruit firmness). The diversity in these fruit traits presents a great opportunity for genetic improvement of blueberry through breeding programmes (selection cycles), especially the traits that are not affected by seasonal variation (e.g. all the traits in our study with the exception of TPH, FRAP, CA and Q). In addition to this, when there is no seasonal variation, some fruit traits could be evaluated at an early stage of selection as found previously [28, 29].

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