Journal of Applied Botany and Food Quality 88, 274 - 287 (2015), DOI:10.5073/JABFQ.2015.088.040

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Nutritional composition of bilberries (*Vaccinium myrtillus* L.) from forest fields in Norway – Effects of geographic origin, climate, fertilization and soil properties

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(Received June 17, 2015)

Summary

Effects of different environmental factors (origin, climate, fertilization and soil properties) on berry nutritional quality were studied in eight forest fields of bilberry (*Vaccinium myrtillus*) in Northern-, Mid- and Southern Norway. No clear trend between locations could be found, however untargeted multivariate analysis of metabolite profiles revealed clear segregation patterns between locations. Anthocyanin, and phenolics content, and titratable acidity were significantly affected by mineral fertilization (Mid-Norway), while organic fertilization did not show any significant effects (Northern Norway). Bilberry chemical composition was affected by harvest time point, as indicated by a potentially higher nutritional quality regarding the content of phytochemicals when harvesting at mid or towards the end of the production season (Southern Norway). Regional and annual climate had strongest impact on the nutritious content of bilberries. Significant differences were found between locations, however previous findings on increasing anthocyanin content with latitude were not confirmed due to environmental impacts confounding the population effects.

Abbreviations

ACY, total anthocyanins; AOX, antioxidant activity; FRAP, ferric reducing antioxidant power; FW, fresh weight; GAE, gallic acid equivalents; GC, gas chromatography; ID, inner diameter; ICA, independent component analysis; MS, mass spectrometry; SS, soluble solids; TA, titratable acidity; TPH; total phenols.

Introduction

The perennial dwarf shrub bilberry (*Vaccinium myrtillus* L.), also called European blueberry, is a member of the Ericaceae family and widely spread in the northern hemisphere across Europe and Central Asia. In comparison to important *Vaccinium* crops such as highbush blueberry (*V. corymbosum* L.) and semi-cultivated lowbush blueberry (*V. angustifolium* Aiton), the food and nutritional quality of bilberry fruits is recognized due to their content of health-beneficial phytochemicals (Moyer et al., 2002). Bilberries are characterized by the high abundance of anthocyanidins and anthocyanins (Kalt et al., 1999) and other potent natural antioxidants such as flavonols and phenolic acids (Lätti et al., 2011), considerable amounts of stilbenes (resveratrol) (Rimando et al., 2004) and ascorbic acid (Moyer et al., 2002). Several studies have shown that berries of *V. myrtillus* contain relatively higher levels of organic acids (Milivojevic et al., 2012), total phenolics (Beccaro et al., 2006; Ochmian et al., 2009; Bunea et al., 2011; Milivojevic et al., 2012), total anthocyanins (Kalt et al., 1999; Beccaro et al., 2006; Ochmian et al., 2009; Bunea et al., 2011) and antioxidants (Halvorsen et al., 2002; Beccaro et al., 2006; Bunea et al., 2011; Milivojevic et al., 2012) compared to cultivated blueberries (*V. corymbosum*). In contrast, soluble solids content is reported to be generally lower in bilberries (Milivojevic et al., 2012). In general, population-specific and varietal differences in concentration levels of distinct phenolic structures such as phenolic acids, flavanols, flavonols, and anthocyanins can be expected, based on findings in bilberry populations (Lätti et al., 2008; Åkerstrom et al., 2010) and cultivated lowbush and highbush blueberries (Moyer et al., 2002; Bunea et al., 2011).

The nutritional quality of bilberries during fruit development is strongly depending on gene expression and activity of enzymes involved in the biosynthesis of flavonols and anthocyanins (Jaakola et al., 2002). Though under strong genetic control, relatively higher anthocyanin levels have been found in bilberries from the northern regions compared to the southern regions of Sweden (Åkerstrom et al., 2010) and Finland (Lätti et al., 2008; Uleberg et al., 2012). The closely related bog bilberry (*V. uliginosum* L.) followed the same pattern with enhanced levels of anthocyanins and flavonols in populations from high latitudes (Lätti et al., 2010). In contrast, altitudinal range seemed to decrease bilberry anthocyanin content (Rieger et al., 2008). Climatic conditions such as temperature, photoperiod and light intensity are important factors affecting berry quality resulting in pronounced year-to-year and within-season variation (Uleberg et al., 2012), revealing fluctuating and transient peak levels of Krebs-cycle-derived organic acids, mono- and di-saccharides, and precursors of phenolic acids. This is also true for flavour compounds in bilberries showing seasonal variation of aroma volatile patterns from early to late season (Rohloff et al., 2009). Grown under controlled conditions, berries produced by northern genotypes showed significantly higher contents of anthocyanins, anthocyanin derivatives, total phenolics, malic acid and sucrose compared to berries of southern genotypes (Uleberg et al., 2012). In addition, lower temperatures resulted in higher levels of flavanols, hydroxycinnamic acids, quinic acid and carbohydrates when cultivating plants at 12 °C compared to 18 °C (Uleberg et al., 2012). Results from this study clearly showed that phytochemical differences are linked to genotypic variation, i.e. the molecular and genetic adaption, and formation of traits depending on environmental conditions plants experience in natural habitats. Such population-specific differences have been shown for berry anthocyanins (Lätti et al., 2008; Åkerstrom et al., 2010) in bilberry, and berry phenolics in bog bilberry (*V. uliginosum* L.) (Lätti et al., 2010) populations. Moreover, genetic markers have been used to characterize the clonal structure of *Vaccinium* sp., and revealed genotypic variation in natural populations of both bilberries (Albert et al., 2003) and lowbush blueberries (Debnath, 2009).

An increasing demand for healthy ingredients by the food industry and changed consumer consciousness have led to investigations of wild berry resources including *V. myrtillus* focusing on genotypic variation, phytochemical content and physiological aspects towards agricultural and industrial exploitation (Nestby et al., 2011). Though light intensity and quality follow natural fluctuations, culturing methods such as forest clear-cutting might improve the plants' ac-

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cess to light and thus, increase biomass production and phenolic content in vegetative tissue of *V. myrtillus* (Nybakken et al., 2013). Soil quality and nutrient availability are on the other hand more easily controllable under potential semi-natural cultivation of bilberry. However, N-fertilization under forest field conditions had no significant effect on bilberry anthocyanin levels compared to the main environmental factors (climate and yearly fluctuations) (Åkerstrom et al., 2009). Soil conditions (Ochmian et al., 2010; Tasa et al., 2012) and application of mineral fertilizers (Lafond and Ziadi, 2011) might positively affect nutrient uptake, vegetative production and yield in *Vaccinium* sp. under cultivation. When cultivating bilberry plants in an agricultural field using different mineral fertilization levels under comparable soil conditions, fruit number was generally increased as an effect of NP fertilization (Nestby et al., 2014a). In contrast, mineral (NP) and organic fertilization (compost or wood chips) to bilberry had negative effects on fruit yield under natural forest field conditions (Nestby et al., 2014b). However, nutritional quality and phytochemical content of blueberries can be improved by soil substrate and fertilization as shown for *V. corymbosum* (Ochmian et al., 2010) and *V. angustifolium* (Albert et al., 2011).

Due to lack of information about the impact of soil characteristics, mineral and organic fertilization on fruit quality parameters of bilberry fruit, experiments in *V. myrtillus* forest fields were carried out at two different field locations in the period from 2008 to 2011. In addition, bilberry populations of different geographic origin (north-south gradient), within-season variation and effects of climate were assessed in order to evaluate the significance of a wide range of environmental factors on bilberry nutritional quality.

Materials and methods

Field descriptions and plant material

Six experimental forest fields were established in Northern-, Mid- and Southern Norway, in addition to two smaller open fields in Northern Norway which served as additional reference fields for Northern Norway (Supplementary Fig. S1). Details of all trial fields regarding location, soil type and forest type are included in Tab. 1. Samples of berry fruits were collected from wild populations of bilberry (*Vaccinium myrtillus* L.). Due to harsh climatic conditions (short summer season, low temperatures), bilberry plants were expected to be derived from clonal (vegetative) propagation, and the age of established plants was estimated to range between 5 to 30 years.

Soil sampling and soil analysis

For a description of soil parameters, experimental design and conditions of forest fields at Sørdalen (F1/F2), Snåsa (F5) and Lierne (F6/F7), refer to the study by Nestby et al. (2014b). An extended list of soil properties (O, E, and B-layer) of all trial fields F1 to F8 is included in Supplementary Tab. S1.

Climate data

Monthly average temperature and precipitation data for growing seasons ranging from April to September for trial years 2008 to 2011 (Supplementary Fig. S2) were collected from nearby meteorological stations (Eklima, 2014): Bardufoss in Målselv municipality (distance to F1/F2 at Sørdalen: 46 km), Harstad municipality (distance to F3 at Stornes: 8 km), Leknes airport in Leknes municipality (distance to F4 at Leknes: 4 km), Kjevlia in Snåsa municipality (distance to F5 at Snåsa: 16 km), Nordli in Lierne municipality (distance to F6/F7 at Lierne: 6 km), and Stavsberg in Ringsaker municipality (distance to F8 at Tveter: 11 km). In order to assess within-season variation of berry quality at field F8 (Tveter) in July and August, weekly average temperature and precipitation data (weeks 27 to 35 from 2008 to 2010) were generated (Supplementary Fig. S3). In addition, data on global irradiance (shortwave) expressed as MJ/m^2, measured at Nes in Ringsaker municipality, was included (distance to F8 at Tveter: 15 km).

Fertilization experiments

Fertilization experiments were performed using mineral fertilization at trial fields F5 (Snåasa) and F6/F7 (Lierne) in Mid-Norway, as described by Nestby et al. (2014b). Due to data consistency, only results from field F5 are particularly presented and discussed. Fields F1/F2 (Sørdalen) in Northern Norway were fertilized using wood chips alone, and wood chips composted with sheep manure (Nestby et al., 2014b). Trial fields F3 (Stornes) and F4 (Leknes) in Northern Norway, and F8 (Tveter) in Southern Norway remained untreated (Tab. 1).

Tab. 1: Description of experimental forest fields in Norway.

Location	Sørdalen	Sørdalen	Stornes	Leknes	Snåsa	Lierne	Lierne	Tveter
Field ID	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Region	North	North	North	North	Mid	Mid	Mid	South
County	Troms	Troms	Troms	Nordland	Nord-Trøndelag	Nord-Trøndelag	Nord-Trøndelag	Hedmark
Municipality	Bardu	Bardu	Harstad	Vestvågøy	Snåsa	Lierne	Lierne	Ringsaker
Altitude (m)	85	85	7	12	297	430	430	290
Coordinates	N68°43.55’ E18°32.68’	N68°44.48’ E18°32.33’	N68°51.23’ E16°28.58’	N68°06.43’ E13°42.26’	N64°18.01’ E12°28.42’	N64°29.99’ E13°29.99’	N64°29.99’ E13°29.99’	N60°49.02’ E10°51.36’
Soil type	silty sand	silty sand	thin soil layer	thin soil layer	silty sand	silty sand	silty sand	loamy sand
Bedrock origin	mica schist	mica schist	mica schist, granite	mica schist, granite	sandstone, clay, granite	sandstone, clay, granite	sandstone, clay, granite	mica schist, gneiss, quarz
Experimental studies	compost, wood chips	compost, wood chips	no treatment	no treatment	mineral fertilization	mineral fertilization	mineral fertilization	within-season variation
Forest type	birch	Scots pine	birch, Norway spruce	birch, shrubs	Norway spruce, birch	Norway spruce	Norway spruce	Norway spruce, birch

Berry sampling and processing

Depending on climate conditions in trial years 2008 to 2011, sampling of fully mature berries (100 to 400 g per plot, whenever possible) was carried out in mid-season in August (F5 at Snåsa and F6/F7 at Lierne; Mid Norway) and end of August/ beginning of September (F1 to F4; Northern Norway). Within-season variation was assessed at trial field F8 (Tveter) in Southern Norway by sampling fully-bluish berries (skin color) from end of July until end of August at 4 time points: A – late July, B – early August, C – mid August, and D – late August. All berry samples were stored frozen at -20 °C prior to sample processing. Twenty-five g of frozen-thawed samples (triplicates) was homogenized with a high-speed homogenizer, and 1 g extracted with 9 mL of 80% methanol for determination of anthocyanins, phenols and antioxidant activity. The remaining homogenate was centrifuged at 3,000 rpm at 4 °C for 15 min; the supernatant (berry juice) was used for measurement of soluble solids, titratable acidity and pH. Berry samples for GC/MS-based metabolite profiling were harvested across trial plots (control) in 2009 from individual plants at locations F5 at Snåsa (n=9), F6 at Lierne and F8 at Tveter (n=10), and F1/F2 at Sørdalen (n=7), kept cold on ice until arrival at the laboratory and further stored at -80 °C prior to sample processing.

Total anthocyanins (ACY)

Total anthocyanins were determined using a modified pH-differential method as described in Uleberg et al. (2012). Buffers of pH 1 (0.025 M) and pH 4.5 (0.4 M) were based on potassium chloride (KCl) and sodium acetate ($C_2H_3NaO_2$). The pH was adjusted with hydrogen chloride (HCl) (all chemicals from Sigma-Aldrich, Germany). Berry raw extracts (see Berry Sampling and Processing) were further diluted in buffer solution whenever necessary, and measured spectrophotometrically at wavelengths 510 and 700 nm. ACY were expressed as cyanidin 3-glucoside per 100 grams of fresh weight (mg/100 g FW).

Total phenolics (TPH)

Total phenolics analysis was based on a modified Folin-Ciocalteu method as described in Uleberg et al. (2012). Berry raw extracts (see *Berry sampling and processing*) were diluted in 80% methanol whenever necessary before incubation at ambient temperature for 2 h. Samples (200 µL) were transferred to a clear 96-well microplate, and absorption measured at 750 nm on a plate reader (Labsystems Multiskan MS, Finland). TPH were expressed as gallic acid equivalents (mg GAE/100 g FW).

Antioxidant activity (AOX)

Berry antioxidants were determined using the ferric reducing antioxidant power (FRAP) assay with some modifications (Uleberg, et al., 2012). Berry raw extracts (see Berry Sampling and Processing) were further diluted in 80% methanol whenever necessary. Samples (5 µL) were transferred to 150 µL FRAP reagent on a clear 96-well microplate, shaken and incubated for 4 min. Absorption was measured at 595 nm on a plate reader (Labsystems Multiskan MS, Finland), and expressed as millimoles of ferric iron reduced (Fe^{2+}) (mM/100 g FW).

Soluble solids (SS)

The soluble solids content was measured in berry juice with a handheld refractometer (ATAGO N-1 E, °Brix 0 to 32% with 0.1% accuracy).

Titratable acidity (TA) and pH

Titratable acidity was determined in a mixture of 5 mL berry juice diluted in 145 ml deionized water by titration with 0.1 M NaOH to an end-point of pH 8.1. Results were expressed as g citric acid (g/100 g FW). The pH was directly measured in the berry juice.

Dry matter (DM)

Dry matter (%) of berry samples (4-5 g of berry homogenate) was determined using a forced-air drying oven at 105 °C overnight until constant weight.

GC/MS-based metabolite profiling

Gas chromatography/mass spectrometry (GC/MS) analysis followed a procedure as described in Uleberg et al. (2012) with some modifications. Samples (2 g) of frozen-thawed berries were mixed with a high-speed homogenizer, and 180 mg of berry homogenate was extracted with 1.8 mL 80% pre-cooled methanol containing ribitol as internal standard (25 µg/mL). Upon centrifugation, an aliquot of 750 µl of extract was used for further sample processing following the method described in Sissener et al. (2011). A Varian Star 3400 CX gas chromatograph coupled with a Varian Saturn 3 mass spectrometer (Walnut Creek, CA) was used for all analyses. 1 µL samples were injected with a split ratio of 25:1 and separated on a HP-5MS capillary column (30 m × 0.25 mm ID, film thickness 0.25 µm). Injection and interface temperature were set at 230 °C and 250 °C, respectively. Helium (He) was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The GC was held isothermically at 70 °C (5 min), ramped from 70 °C to 310 °C (5 °C min^{-1}), and finally held at 310 °C (7 min). MS source temperature was adjusted to 230 °C and a mass range of *m/z* 50-550 was recorded (EI mode). GC/MS data integration, normalization (total signal) and alignment was carried out in an untargeted approach, using the *MetAlign* software (Vers. 041011) for full scan MS comparison developed by PRI-Rikilt, Wageningen, The Netherlands (Lommen, 2009). Aligned data was filtered by selecting individual MS fragments with highest intensity from detected metabolite peaks. The total number of 817 non-annotated MS metabolite tags which could be detected in all 36 samples, was used. Data was further normalized to the internal standard and finally, log2(n) ratios were calculated based on median values of each metabolite tag prior to statistical analysis.

Statistical analysis

Multivariate statistical analysis of GC/MS profiling data was carried out using independent component analysis (ICA) via the web-based tool *MetaGeneAlyse* available at the Max Planck Institute of Molecular Plant Physiology (MPI-MP), Golm, Germany (Scholz et al., 2004). For statistical evaluation of geographic origin, year-to-year variation, organic fertilization (F1/F2 at Sørdalen) and mineral fertilization (F5 at Snåsa), and effects of harvest time on within-season variation (F8 at Tveter), ANOVA was carried out in combination with Fisher's LSD test using 95% individual confidence intervals (Minitab® software v.17.1.0) based on replicate data from all single measurements (Tab. 2 and 3; Fig. 2). Pearson correlation analysis (Microsoft® Excel 2010) was used to indicate potential impact of soil (data from 2009) and climate factors (annual data) on berry quality (annual means) from corresponding field trial locations (Supplementary Tab. S2). Furthermore, Pearson correlation analysis was carried out to show relationships between quality parameters of berry samples based on replicate data from single measurements, either using data from all trial fields combined (Supplementary Tab. S3) or measurements from different harvest time points at field F8 (Tveter) (Supplementary Tab. S4).

Tab. 2: Berry quality at different field locations based on corresponding measurements of total anthocyanins (ACY), total phenols (TPH), antioxidant activity (AOX), soluble solids (SS), titratable acidity (TA), pH and dry matter (DM) as averages of the years 2008 to 2011. Letters indicate significant differences ($P < 0.05$) between locations.

	Location					
Quality Papameter	**F1/F2 Sørdalen**	**F3 Stornes**	**F4 Leknes**	**F5 Snåsa**	**F6/F7 Lierne**	**F8 Tveter**
ACY (mg/100 g FW)	358^{bc}	447^{a}	383^{abc}	417^{a}	330^{c}	385^{ab}
TPH (mg/100 g FW)	603^{b}	674^{a}	606^{ab}	598^{b}	606^{b}	531^{c}
AOX (mM/100 g FW)	6.74^{b}	7.37^{ab}	6.89^{ab}	7.82^{a}	7.58^{ab}	4.96^{c}
SS (°Brix)	9.85^{b}	8.24^{c}	10.13^{b}	11.01^{a}	10.14^{b}	8.93^{c}
TA (g/100 g FW)	1.23^{b}	1.14^{bc}	1.26^{b}	1.23^{b}	1.40^{a}	1.09^{c}
pH	2.87^{a}	2.79^{bc}	2.76^{c}	2.81^{c}	2.68^{d}	2.85^{ab}
DM (%)	16.11^{a}	13.62^{bc}	12.79^{c}	15.90^{a}	14.90^{ab}	13.33^{c}

Tab. 3: ANOVA analysis (Fisher's test; 95% individual confidence interval) of effects of geographic origin, organic and mineral fertilization, harvest time (within-season variation) and year-to-year variation on berry quality parameters. Statistical calculations are based on corresponding measurements from years 2008 to 2011.

Trial field:	**F1 – F8**		**F1/F2 – Sørdalen**		**F5 – Snåsa**		**F8 – Tveter**	
Quality parameter	**Origin**	**Year**	**Organic Fertil.**	**Year**	**Mineral Fertil.**	**Year**	**Harvest Time**	**Year**
ACY	0.001	<0.001	ns	0.026	0.001	0.064	0.015	<0.001
TPH	<0.001	<0.001	ns	ns	0.004	0.007	ns	<0.001
AOX	<0.001	<0.001	ns	<0.001	ns	<0.001	0.285	<0.001
SS	<0.001	<0.001	ns	ns	ns	<0.001	<0.001	<0.001
TA	<0.001	<0.001	ns	ns	0.117	<0.001	0.387	<0.001
pH	<0.001	<0.001	ns	0.044	ns	0.001	<0.001	<0.001
DM	<0.001	<0.001	ns	ns	ns	0.021	<0.001	<0.001

ns = not significant

Results and discussion

In the following, results from analyses of *V. myrtillus* are presented in relation to geographic origin and populations from all field trial locations (Supplementary Tab. S1), regardless of seasonal variation, climatic conditions and plant nutritional aspects, in order to emphasize natural variation in chemical composition of Norwegian bilberries. Subsequently, the impact of organic (trial fields F1/F2 at Sørdalen) and mineral fertilization (trial field F5 at Snåsa), soil properties and climate, and effects of harvest time point (trial field F8 at Tveter) on nutritional quality of bilberries are discussed. Climate data (temperature and precipitation) are presented and discussed in combination with results whenever necessary.

Geographic origin and population influence berry quality

Mature bilberries were harvested at 6 locations (8 trial field plots) in Northern, Mid- and Southern Norway. Berry samples were subjected to chemical analyses in order to assess quality differences based on several parameters (Tab. 2). The values indicated strong effects of population origin and potentially genotype on berry quality. The highest average ACY levels were observed at trial fields F3 at Stornes (7 masl) and F5 at Snåsa (297 masl) differing significantly from locations F1 (85 masl) and F2 (85 masl) at Sørdalen, and F6/F7 (430 masl) at Lierne – which grew either far north or at higher altitude (>400 masl). The field at F3 (Stornes) also produced berries with the highest phenol content (TPH) compared to the other locations, with the lowest concentrations observed in berries from Tveter (F8) in Southern Norway. In addition to low TPH, berries from F8 were also significantly lowest in AOX levels followed by F1/F2 (Sørdalen), while berries from Snåsa (F5) had higher levels than those from F1/F2, but could not be separated from berries at F3 (Stornes), F4 (Leknes) and F6/F7 (Lierne). In addition, a relative strong correlation between TPH and AOX levels was found across all locations, while the relationship between ACY and AOX was much weaker, thus underscoring the significance of phenolic compounds other than anthocyanins, in their contribution to overall antioxidant activity in bilberries (Supplementary Tab. S3 and S4). Soluble solids content (SS) was highest at F5 (Snåsa) and differed significantly from all other locations with lowest levels in berries from F3 (Stornes) in Northern Norway and at F8 (Tveter) in Southern Norway. Also TA levels were clearly lowest at F8, while the highest levels were observed at Lierne in Mid Norway. pH levels were negatively correlated with TA, and found significantly different with lowest values at location F6/F7 (Lierne) in comparison with the other trial fields, except F8 (Tveter). Bilberries from Sørdalen (F1/F2) and Snåsa (F5) had highest DM content throughout the trial years, but could not be statistically distinguished from F6/F7. In contrast, berries from Leknes (F4) and Tveter (F8) showed the lowest contents. No clear trend between locations could be found, which might indicate one or several factors to be responsible for variation in berry quality. However, the trial field in Southern Norway (F8 at Tveter) showed both lowest TPH and AOX levels of the berries, and also differed significantly negatively from many other fields, in the case of SS, TA, pH and DM. It is interesting though, that the northern fields F3 (Stornes) and F4 (Leknes) were high in ACY, TPH and AOX. Typical for these fields was their coastal location situated approximately 10 masl, while the third northern experimental area at Sørdalen (F1/F2) showing relatively low values, was continental and located at a much higher altitude and thus, was comparable to F5 (Snåsa) in Mid Norway.

In addition, GC/MS-based metabolite profiling was conducted in an untargeted approach to characterize differences in chemical profiles of extracted sugars and organic acids of bilberries from locations F1/F2 (Sørdalen), F5 (Snåsa), F6 (Lierne) and F8 (Tveter). Based on a total of 817 detected and non-annotated metabolite tags found in all samples, data was subjected to multivariate analysis (Fig. 1). ICA revealed clear segregation patterns of samples from Southern (F8 at Tveter), Mid (F5 at Snåsa) and Northern Norway (F1/F2 at Sørdalen), while bilberries from trial field F6 (Lierne) were distributed among all other samples. Compared to the otherwise gathered quality parameters, GC/MS separation of derivatized berry extracts potentially covers a wide range of different chemical structures including carbohydrates, sugar acids and – alcohols, Krebs cycle acids, amino acids and amines, fatty acids, glycerides, sterols, simple phenols and partly polyphenols. Derived information of non-annotated MS tags from these analyses showed a relatively high degree of variation. However, the observed effects of geographic origin and population on sample variation (Fig. 1) are in line with results from statistical analysis of quality parameters (Tab. 2 and 3), showing clear differences between bilberries from Southern (F8 at Tveter), Mid (F5 at Snåsa) and Northern Norway (F1/F2 at Sørdalen).

Results from the present study are in accordance with earlier findings on berry samples from natural populations of *V. myrtillus* showing similar levels of ACY (330 to 447 mg) (BECCARO et al., 2006; LÄTTI et al., 2008; BUNEA et al., 2011), TPH (531 to 674 mg) (BECCARO et al., 2006; BUNEA et al., 2011), and AOX (4.96 to 7.82 mM) (HALVORSEN et al., 2002; BECCARO et al., 2006). Several studies have emphasized the impact of geographic origin (RIEGER et al., 2008; BUNEA et al., 2011; MIKULIC-PETKOVSEK et al., 2015), latitude and potentially light conditions (LÄTTI et al., 2008; ÅKERSTROM et al., 2010; MIKULIC-PETKOVSEK et al., 2015) and light quality, also in combination with genotype (ULEBERG et al., 2012) on berry quality. As pointed out by JAAKOLA and co-workers (2002), anthocyanin biosynthesis in bilberry underlies genetic and fruit-developmental control and is strongly determined by the genotype (ULEBERG et al., 2012). However, distinct north-south latitudinal effects on ACY and other quality parameters related to phenolic constituents (LÄTTI et al., 2008; ÅKERSTROM et al., 2010), also reported for other *Vaccinium* species (LÄTTI et al., 2010), could not be confirmed by our data (Supplementary Tab. S2), not least because the impact of origin and population was strongly masked by year-to-year variation (Tab. 3). In contrast, other quality parameters such as SS observed in highbush blueberry, might not necessarily be depending on climate conditions varying from year to year (REMBERG et al., 2006). When harvesting bilberries at closely-located plots thus limiting climate, topography, and soil effects, no significant differences in profiles of polyphenols in relation to habitat and light conditions were revealed (ELISABETTA et al., 2013). Such homogeneity of within-population fruit quality is therefore also reflected by results from metabolite profiling and ICA analysis (Fig. 1).

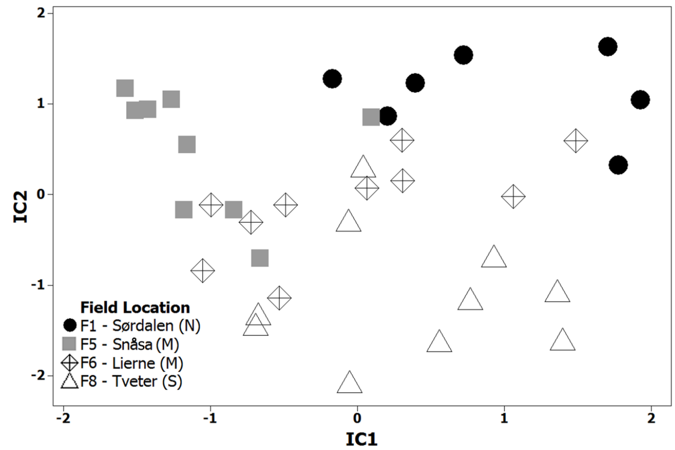

Fig. 1: Independent Component Analysis (ICA) in an untargeted approach based on a total of 817 non-annotated metabolite tags of berry samples from individual plants harvested at field trial locations F1 (Sørdalen) (n=7), F5 (Snåsa) (n=9), and F6 (Lierne) and F8 (Tveter) (both n=10) in 2009. N = Northern, M = Mid, and S = Southern Norway.

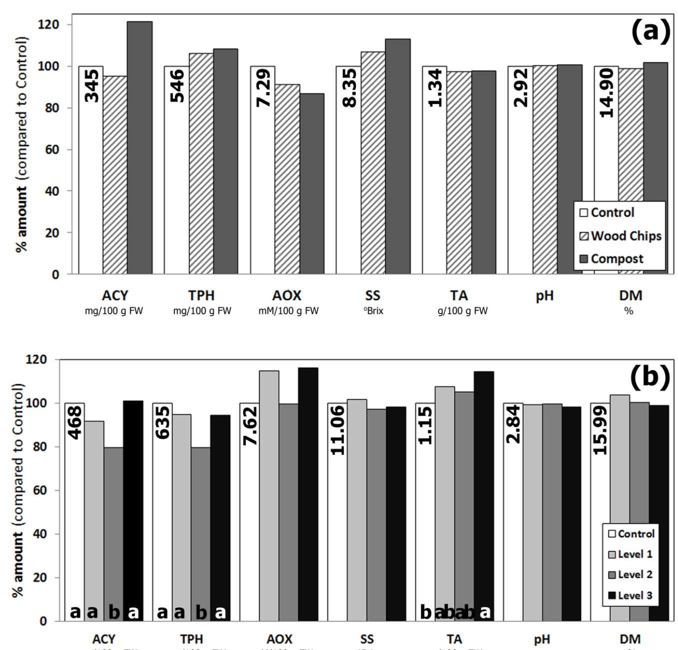

Fig. 2: Quality parameters (mean values from 2009 to 2011) in bilberries upon fertilization. (**a**) Organic fertilization (wood chips and compost) at trial fields F1/F2 (Sørdalen) in Northern Norway. (**b**) Mineral fertilization at trial field F5 (Snåsa) in Mid Norway: Control – 0 kg N/ha; Level 1 – 30 kg N and 20 kg P/ha; Level 2 – 30 kg N and 40 kg P/ha; and Level 3 – 60 kg N and 40 kg P/ha. Letters indicate significant differences ($P < 0.05$) between treatments, based on corresponding measurements (replicate data from all trial years).

Effect of climate and soil properties

Highest average temperatures throughout the growth period from April to August were observed at the most southern field (Tveter, Supplementary Fig. S2). In addition, summer temperatures in 2010 were comparably low from April to July at fields in Southern and Mid Norway. In contrast, the location at Sørdalen showed relatively high temperature differences both in June and August between trial years. Moreover, trial year 2010 was characterized by relatively higher precipitation levels particularly in Northern Norway (June and July), whereas the rainiest months close to or above 150 mm pr. month in July or August were observed at fields F6/F7 at Lierne (2010 and 2011) and at F8 at Tveter in the south (2008 and 2009). The differing climate conditions might explain variation in year-to-year berry quality (ÅKERSTROM et al., 2009; ULEBERG et al., 2012), as underscored by ANOVA statistical analysis (Tab. 3). However, no clear conclusions could be drawn from Pearson correlation of quality measurements with climate data such as monthly average temperature, heat sum and aggregated heat sum (April to August), and precipitation (March to August) (Supplementary Tab. S2). At least higher daily temperatures (heat sum in July and August) at Tveter (F8) might explain the relatively lower levels of both TPH, AOX, SS and TA (negative correlation), and thus partly confirm earlier findings on temperature effects (18 vs. 12 °C) on phenolic compounds and sugar levels (ULEBERG et al., 2012). Regarding effects of precipitation, a decrease of soluble solids and increase of titratable acidity was observed in field experiments with irrigation to lowbush blueberry plants (GLASS et al., 2005). In comparison, no relationship between rainfall and taste quality (SS and TA) was found in our study. However, a positive correlation between precipitation (probably as snow) in March/April and AOX and ACY (April) levels could be shown, while increased precipitation in July/August was negatively linked to phenolic contents (Supplementary Tab. S2). To which extent temperature and precipitation directly affect berry quality parameters cannot easily be deduced from the comprehensive data set and thus, will be further discussed in context with harvest time point effects recorded at trial field F8 (Tveter).

Soil properties are naturally strongly influenced by local and regional climate conditions. The factors light, temperature, precipitation and mineral composition of the original bedrock have impact on pedogenesis and establishment of plant populations. Degradation and humification of organic material from the vegetation in turn strongly influences the C and N balance, mineral composition, acidity and field capacity in soils, and thus add to the variability of growth conditions found in natural habitats of *V. myrtillus*. A systematic analysis of soil properties at all locations revealed strong differences with regard to distinct characteristics of O-, E- and B-layers, as already discussed for trial fields F1/F2 (Sørdalen), F5 (Snåsa) and F6/F7 (Lierne) (NESTBY et al., 2014b).

However, little is known about effects of local soil properties on quality parameters in bilberry fruit. In our field trials it could be shown that a thick O-layer was positively correlated with TPH, AOX, SS, TA and DM, and thus confirm the importance of this layer with regard to berry metabolism (Supplementary Tab. S2). Moreover, a high amount of silt in the B-layer was positively correlated with soluble solids content (SS). The positive effect of silt vs. sand might be explained as a result due to favourable water and nutrient capacity, while clay had negative effect on almost all quality parameters. Generally, increased levels of macronutrients negatively affected all important quality parameters. Moreover, results from bilberry cultivation under agricultural field conditions with mineral fertilization suggest that accumulation of nutrients throughout several years reduced bilberry yield (NESTBY et al., 2014a). However, whether N or P had the strongest negative effect could not be deduced from these experiments. In general, most of the roots are growing throughout the organic O-layer, and it is likely that potential fertilizer effects are most pronounced here. NH_4^+ ions will accumulate in the O-layer because of adsorption to organic functional groups, while negative charged P ions will be leached to the E and B layers and bound by Fe and Al. Nutrient leaching is strongly influenced by precipitation, i.e. over time leached P will be less available for plant uptake. Because of soil chemical binding processes it is likely that negative effects of the strongest fertilization levels are a result of potential excessive fertilization and mainly linked to N accumulation in the O-layer.

Effect of organic fertilization

Nutrient availability from organic fertilizers is generally based on a slow-release effect, depending on the type of organic matter applied and its degree of humification or fermentation. Here we compared two types of organic material, wood chips alone or compost (wood chips composted with sheep manure), and their effects on fruit quality of bilberries harvested at field F1/F2 (Sørdalen) in Northern Norway. Despite relatively high variation between treatments and non-significant results (Tab. 3), trends of increasing levels of ACY, TPH and SS and decreasing antioxidant activity in berries from fertilized plants could be observed based on mean values from the 3-years trial period (Fig. 2a). In contrast to fields F5 (Snåsa) in Mid Norway and F8 (Tveter) in southern Norway, significant year-to-year effects at Sørdalen field trial location could only be observed for parameters ACY, AOX and pH. Thus, the obvious increased ACY levels in berries from compost-treated plots might be explained by strong variations between years. In comparison to earlier studies, only few reports describe the impact of organic fertilization on yield and quality in *Vaccinium* sp. growing in natural habitats. Recent results from agricultural field experiments with *V. myrtillus* in Mid Norway revealed potential positive long-term effects of top-dressing (natural peat) on fruit number pr. plant (NESTBY et al., 2014a), while bilberry yield measurements from the Sørdalen field (F1/F2) (NESTBY et al., 2014b) indicated even a yield decrease when adding organic matter. In a study with lowbush blueberry, low-level organic fertilization (papermill sludge) was shown to increase yields (GAGNON et al., 2003), based on an improvement of properties of the low fertility soil. Growth and yield enhancing effects have also been demonstrated for highbush blueberry cultivars when applying pine bark (PLISZKA et al., 1997) and different types of organic fertilizer such as pine needle, manure and compost (BURKHARD et al., 2009). Berry quality on the other hand has only been addressed in a few studies. ECHEVERRÍA and co-workers (2009) could not find any significant effects of organic fertilization (manure, compost and waste products) on highbush blueberries regarding parameters SS, TA and pH, while other studies reported decreased SS levels (pine needle and manure/sawdust compost) (BURKHARD et al., 2009). Moreover, the content and composition of health-beneficial compounds (anthocyanins and antioxidant capacity) in *V. corymbosum* might be differently affected depending on the used substrate type for plant cultivation (peat, sawdust and cocoa husk) (OCHMIAN et al., 2010). Cultivation of highbush blueberry plants on farm land using additional pine bark mulching, resulted in relatively lower levels of berry phenolics and AOX (EICHHOLZ et al., 2011). Moreover, BURKHARD and co-authors (2009) did not observe any significant differences between treatments in TPH when investigating various types of organic mulch applied to *V. corymbosum*. In conclusion, results from the present study showed that organic fertilization had minor effects on quality parameters in bilberry fruits, similar to earlier reports from other *Vaccinium* crops under semi-cultivation (*V. angustifolium*) or field cultivation (*V. corymbosum*).

Effect of mineral fertilization

Mineral fertilization, in particular nitrogen application, has been shown to have positive impact on vegetative growth and fruit set and yield in *V. myrtillus* (NESTBY et al., 2014a) and cultivated *Vaccinium* sp. (PLISZKA et al., 1997; LAFOND et al., 2011), while anthocyanin biosynthesis might be negatively affected as shown for bilberries (ÅKERSTROM et al., 2009). In the present study, different levels and combinations of N and P fertilizers varying from 0 to 60 and 0 to 40 kg ha^{-1}, respectively, were tested in their effect on berry quality at trial location F5 (Snåsa) in Mid Norway. Among the measured parameters, anthocyanin and phenolics content, and titratable acidity were significantly affected compared to the control group (Tab. 3) with highest TA levels upon fertilization at NP-level 3 (Fig. 2b). Generally, fertilizer application at level 1, and level 2 in particular reduced ACY and TPH content, while all other parameters (SS, pH and DM) were relatively unaffected. On the other hand, strong significant differences for all parameters were observed between the trial years (Tab. 3). Except for fertilization level 3, our results are in accordance with ÅKERSTRØM et al. (2009), who found no significant effect of N fertilization on anthocyanidin levels in bilberry, but a rather strong impact of climatic factors. N application alone has been shown to increase spatial growth and fruit number, while P not necessarily had a specific effect (though growth enhanced when combined together with N) (NESTBY et al., 2014a). Several studies using mineral fertilization reported effects on the yield structure in *Vaccinium* sp., i.e. modification of individual berry weight and area yield. Yield enhancing effects of N and NPK fertilization in bilberries (NESTBY et al., 2014a), lowbush (LAFOND et al., 2011) and highbush blueberries (PLISZKA et al., 1997) have been observed. To which extent mineral nutrition, either applied in natural habitats or agricultural fields, affects nutritional quality of *Vaccinium* berries has only been scarcely addressed. In the Swedish study from 2009 investigating bilberry anthocyanin content and composition (ÅKERSTROM et al., 2009), ACY levels were slightly (not significantly) increased upon N fertilization using two levels of N fertilization (12.5 and 50 kg/ha). Regarding metabolism of vegetative tissue, additional NPK fertilization was shown to affect individual compound levels of phenolic acids, flavonols and condensed tannins in *V. myrtillus* leaves (NYBAKKEN et al., 2013). Application of NPK fertilizer to lowbush blueberry at lower levels (N level: 8 and 24 kg/ha) resulted in higher berry sweetness (SS/TA ratio) based on relatively reduced TA levels compared to high N levels (56 kg/ha) (ALBERT et al., 2011). Moreover, NPK application at lowest level resulted in decreased content of ACY and AOX, but results were not coherent with regard to increasing N levels. However, in combination with top dressing (liming) ACY contents were enhanced in all trial years and at all N levels compared to control plants. In a trial with two highbush blueberry cultivars levels of TPH and AOX decreased with higher N fertilization (EICHHOLZ et al., 2011). In sum, based on our own data and partly contrary experimental results from other studies in different *Vaccinium* species, potential positive effects of mineral fertilization on metabolism of berry phytochemicals in *V. myrtillus* could not be deduced.

Effect of harvest time point

Growth and performance of bilberry plants is clearly affected by light conditions provided by the surrounding ground and tree vegetation (NESTBY et al., 2011). Furthermore, potential effects of photoperiod (LÄTTI et al., 2008; ÅKERSTROM et al., 2010) and light quality (ULEBERG et al., 2012) on berry composition have recently been described. In order to assess the impact of seasonal variation of temperature, precipitation and irradiance on fruit quality under natural growth conditions, mature berries were sampled at field F8 at Tveter in Southern Norway throughout the growing season from the end of July until end of August. Strong significant differences between harvest time points A to D were observed for the measured parameters anthocyanins, soluble solids, pH and dry matter (Tab. 4), thus reflecting earlier observations by Åkerstrøm et al. (ÅKERSTROM et al., 2009). Moreover, berry quality was strongly affected by year-to-year-variation. Differences in climate conditions from 2008 to 2010 (Supplementary Fig. S3) are likely responsible for berry quality variation of all parameters. Further correlation analysis of berry quality with climate data for the corresponding harvest time points revealed relatively stronger effects based on the climate factors temperature and global irradiance (Tab. 3). In contrast, precipitation seemed to negatively influence parameters such as SS, TA, DM and also TPH, however effects were not that strongly pronounced. SS, TA and DM content were positively linked to temperature and irradiance during the sampling week and the preceding week(s), while pH levels were negatively affected. In comparison, elevated irradiance, also of preceding weeks prior to harvest, seemed to negatively influence berry anthocyanin levels. These observations were further underscored by a negative relationship (Pearson correlation) of ACY with SS, TA and DM levels (Supplementary Tab. S4), indicating a competitive relationship between primary metabolism (sugars and acids) and secondary metabolism (anthocyanins). Based on mean values from the 3-years trial period, both ACY, AOX, pH and TPH showed increasing levels towards the end of the sampling period (end of August), while levels of SS, TA and DM were decreasing (Fig. 3). In addition, a calculation of phenolic constituents and antioxidants on a dry matter basis (data not shown) revealed the same trends, and thus verified that measurements were not skewed due to varying berry moisture content. In comparison with studies focusing on ripening berries from the green to the overripe stage showing clear increases of SS content in e.g. lowbush blueberry (GIBSON et al., 2013), here we focused on the edible quality of mature bilberries harvested at different time points. In view of enhancing effects of thermal sum on anthocyanin levels during the growing season (ÅKERSTROM et al., 2009), the present results corroborate such findings. Bilberries having perceived a higher thermal sum and being harvested at later time points, showed higher levels of phenolic constituents indicated by ACY, TPH and AOX data (Fig. 3). Thus, a potentially higher nutritional quality of berries regarding the overall content of phytochemicals might be achieved when harvesting at mid or towards the end of the production season.

Tab. 4: Pearson correlation of berry quality data at different harvest times (week 30 – A, week 31 – B, week 32 – C, week 33 – D; see Materials and methods) from 2008 to 2010 and corresponding weekly climate data for average temperature, aggregated temperature sum, sum precipitation and average global irradiance. In addition, quality measurements were correlated with climate data of the respective preceding week(s) (-1w = 1 week before; -2w = 2 weeks before; -3w = 3 weeks before).

Correlation	**Temperature** °C				**Aggreg. Temperature** °CΣ				**Precipitation** mm				**Global Irradiance** MJ/m^2			
	0w	-1w	-2w	-3w	0w	-1w	-2w	-3w	0w	-1w	-2w	-3w	0w	-1w	-2w	-3w
ACY	-0.250	-0.040	-0.376	-0.248	-0.145	-0.104	-0.121	-0.126	-0.167	0.079	0.088	0.498	-0.409	-0.203	-0.596	-0.701
TPH	0.087	0.020	-0.142	-0.091	0.533	0.494	0.490	0.557	0.232	-0.428	0.129	0.263	0.259	0.160	-0.227	0.222
AOX	-0.305	-0.226	-0.184	-0.175	0.426	0.401	0.442	0.504	0.049	-0.136	0.003	0.490	0.077	-0.153	-0.349	-0.124
SS	0.534	0.441	-0.081	-0.138	-0.182	-0.254	-0.292	-0.259	0.252	-0.454	-0.526	-0.172	0.548	0.738	0.384	0.391
TA	0.591	0.124	0.258	0.198	-0.050	-0.104	-0.133	-0.143	0.135	-0.178	-0.031	-0.457	0.522	0.306	0.426	0.685
pH	-0.730	0.092	0.136	-0.179	0.494	0.556	0.571	0.536	0.062	0.277	0.088	0.497	-0.725	-0.370	-0.196	-0.598
DM	0.792	0.430	-0.360	-0.134	-0.273	-0.345	-0.379	-0.349	0.051	-0.652	-0.365	-0.112	0.679	0.771	0.215	0.342

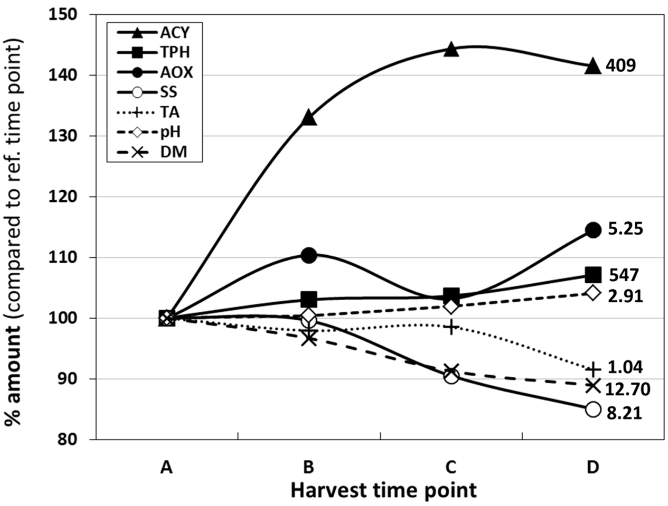

Fig. 3: Quality of mature berries sampled at location F8 (Tveter) in Southern Norway. Curves depict within-season variation (% amount) at different harvest times based on average values from years 2008 to 2010. Mean values of quality parameters measured are quoted for the last time point (ACY and TPH in mg/100 g FW; AOX in mM/100 g FW; SS in °Brix; TA in g/100 g FW; DM in %). Time points: **A** – late July (reference), **B** – early August, **C** – mid August, and **D** – late August.

Conclusion

In general, a comprehensive and simultaneous screening of multiple berry quality parameters, considering the significance of plant origin, harvest time and year, nutrition and soil properties has been lacking so far. The present study has provided new insights into different factors affecting bilberry nutritional quality. Environmental effects of regional and annual climate had strongest impact on bilberry quality. The results revealed strong differences in patterns between regions, but previous findings concerning latitudinal effects were not confirmed, probably because the environmental impacts confounded the genetic (population) effects. Fertilizing by mineral fertilizers affected quality, but organic fertilization did not show significant effects. This may be due to the relatively lower nutrient availability of the applied organic fertilizers as these may be expected to have more long-term effects. Harvest time affected quality and in general, harvesting mid or late season enhanced the nutritional quality of bilberry due to higher levels of health-beneficial phytochemicals and increased antioxidant activity.

Acknowledgements

This study was funded by The Research Council of Norway MATPROGRAMMET through the project "Cultivation of the European blueberry" grant no. 184797 to Bioforsk Nord (now: NIBIO), and through private funding from active project participants and industry: Anne Britt Hanstad, Viltost fra Snåsa, Lierne Næringsselskap AS, Pihl AS, AS Røra Fabrikker, and Tine BA. We are grateful to Aud and Per Sandnes (Snåsa), Arnstein Kirste (Lierne) and Anne Britt Hanstad (Bardu) for providing forest fields and help with berry sampling, and Grete Rakvaag (Dep. Biology, NTNU), and master student Eva S. Dahlø (Dep. Biology, NTNU) for analytical and technical assistance.

Supplementary data

Supplementary Tab. S1. Overview of soil characteristics of all trial fields. **Supplementary Tab. S2.** Pearson correlation analysis of climate (annual data) and soil factors (2009) with berry quality measurements (annual means) from corresponding field trial locations. **Supplementary Tab. S3.** Pearson correlation between quality parameters of berry samples based on replicate data from single measurements (all trial fields). **Supplementary Tab. S4.** Pearson correlation between quality parameters of berry samples based on replicate data from single measurements at different harvest time points at field F8 (Tveter). **Supplementary Fig. S1.** Trial field locations in Norway. **Supplementary Fig. S2.** Monthly average temperature and precipitation data for all field locations throughout the growing season (April to September) from 2008 to 2011. **Supplementary Fig. S3.** Weekly average temperature, precipitation and global irradiance for field F8 (Tveter) in Southern Norway throughout the summer season in July and August (weeks 27 to 35) from 2008 to 2010.

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Supplementary Tab. S1: Overview of soil characteristics of all field trials. Data partly reprinted from Nestby et al. (2014b), J. Berry Res., 4, 84-85, with permission from IOS Press (©2014).

Location	Layer	DM (%)	Layer (cm)	Vol.W. (g/L)	clay	silt	sand	Ignit. loss (%)	$C_{Tot.}$ (%)	$N_{Tot.}$ (%)	pH	Ca_{Al} (mg/kg)	K_{Al} (mg/kg)	Mg_{Al} (mg/kg)	Na_{Al} (mg/kg)	P_{Al} (mg/kg)	P_{ox} (mg/kg)	Fe_{ox} (mg/kg)	Al_{ox} (mg/kg)
Sørdalen	**0h**	96.1	5.9	0.19				42.7	26.3	1.0	4.37	1518	561	438	37.5	129			
(F1)	**E**	99.3	8.3					3.7			4.13	78.7	32.4	30.0	6.4	8.9	62.5	1800	639
	B	98.6			3.7	35.4	60.9	4.7			4.49	44.6	15.8	21.0	6.7	5.2	190	12688	2813
Sørdalen	**0h**	94.6	7.3	0.14				68.0	41.1	1.1	4.10	1482	569	440	75.4	140			
(F2)	**E**	99.5	8.1					2.7			4.11	52.0	20.0	17.8	7.9	5.4	31.7	692	617
	B	98.9			2.6	37.2	60.3	3.5			4.46	49.3	9.4	19.8	7.2	4.3	133	8450	2367
Stornes	**0h**	93.3						83.1			4.06	909	452	602	231	156			
(F3)	**B**											202	119	195	107	29.7			
Leknes	**0h**	96.8						33.0			4.25	395	332	404	117	112			
(F4)	**B**	98.9						9.2			4.50	57.1	96.4	75.6	32.2	35.0			
Snåsa	**0h**	95.5	7.8	0.22				51.7	30.6	1.0	4.05	886	410	359	62.0	103			
(F5)	**E**	99.6	5.5					2.6			4.25	56.3	16.2	18.6	5.6	4.6	22.0	1670	469
	B	97.3			6.7	48.9	44.5	9.7			4.44	89.3	22.7	24.1	8.7	6.5	129	23050	6250
Lierne	**0h**	97.3	4.6	0.35				28.1	16.5	0.6	4.49	825	319	291	32.2	78.1			
(F6)	**E**	99.4	5.9					3.2			4.44	80.0	18.4	25.9	6.4	7.2	52.0	3550	870
	B	98.0			4.9	36.5	58.6	6.2			4.67	60.8	26.9	17.8	6.9	5.3	197	21190	5580
Lierne	**0h**	93.2	9.3	0.22				57.1	32.4	1.1	4.13	1335	445	465	40.5	114			
(F7)	**E**	99.2	4.6					4.8			4.17	79.5	28.0	32.0	8.7	9.2	47.5	2750	641
	B	97.2			6.1	42.5	51.5	9.9			4.54	120	28.2	39.8	6.3	8.6	131	25538	5188
Tveter	**0h**	96.2	3.5	0.33				44.1	25.8	0.9	4.21	1179	528	187	8.6	141			
(F8)	**E**	98.3	3.3					9.1			3.98	193.6	75.1	32.6	3.0	31.7	238	3625	2063
	B	98.5			16.0	39.0	45.0	5.8			4.38	88.9	51.3	16.0	2.3	24.4	275	6163	2850

Supplementary Tab. S2: Pearson correlation analysis of climate (annual data) and soil factors (2009) with berry quality measurements (annual means) from corresponding field locations.

FACTOR		ACY	TPH	AOX	SS	TA	pH	DM
Latitude		0.0853	0.3766	0.1680	-0.1505	0.1302	0.0633	0.0127
Temperature °C	Apr	0.1457	-0.3141	0.0890	0.0036	-0.3797	0.0740	-0.2420
	May	0.0015	-0.3861	-0.1315	-0.2853	-0.4187	0.2007	-0.3962
	Jun	-0.1943	-0.3038	0.2801	0.2388	-0.0325	-0.0522	0.2056
	Jul	-0.1277	-0.4304	-0.0632	0.1250	-0.0408	-0.0474	-0.0372
	Aug	0.1092	-0.3379	0.2487	-0.0335	-0.3174	-0.2365	-0.2282
Heat Sum °CΣ	Apr	0.0471	-0.3168	-0.1666	-0.2460	-0.5092	0.2445	-0.2640
	May	-0.0365	-0.3886	-0.3369	-0.3648	-0.4955	0.3146	-0.3251
	Jun	-0.0965	-0.3278	-0.1243	-0.1195	-0.2196	0.1621	-0.0960
	Jul	-0.1045	-0.4703	-0.4703	-0.1419	-0.1968	0.1189	-0.2058
	Aug	0.2237	-0.4698	-0.1714	-0.3727	-0.5729	0.2380	-0.3117
Aggregated	Apr	-0.1765	-0.2391	0.0840	0.0653	-0.2521	-0.0204	-0.2704
Heat Sum °CΣ	May	-0.1378	-0.2998	0.0170	-0.0591	-0.4269	0.0807	-0.2761
	Jun	-0.2235	-0.4209	0.0437	0.0430	-0.2097	0.0938	-0.0440
	Jul	-0.2331	-0.4434	0.0306	0.0836	-0.1847	0.0611	-0.0089
	Aug	-0.1771	-0.4445	0.0722	0.0751	-0.2283	0.0113	-0.0037
Precipitation mm	Mar	0.2000	0.0694	0.6172	0.2230	0.2029	0.1668	0.3273
	Apr	0.4217	-0.0598	0.4964	-0.0708	-0.1726	-0.0287	-0.1375
	May	-0.1394	-0.1069	0.1442	0.2211	-0.1302	-0.1537	-0.0522
	Jun	0.1791	-0.2074	-0.2362	0.1480	0.2559	0.3312	0.2670
	Jul	0.3779	-0.5214	-0.3951	-0.3488	-0.3299	0.5263	-0.2047
	Aug	-0.1629	-0.4004	0.2956	0.1097	0.0703	0.0345	0.0998
Loss on ignition %	Oh	0.3325	0.0793	0.1813	-0.3837	-0.0829	0.2727	0.0390
	Eh	0.0078	-0.3554	-0.4002	-0.4401	-0.3906	0.0752	-0.5558
	Bh	0.0454	0.2879	0.1921	0.5159	-0.0642	-0.5163	-0.0716
Layer size cm	Oh	-0.0057	0.2396	0.4301	0.4689	0.4414	-0.1956	0.4766
	Eh	-0.0338	0.2860	0.1675	0.0356	0.2839	0.2930	0.4731
Total C %	Oh	0.1232	-0.4301	0.0583	-0.2604	0.3565	0.5222	0.1146
Total N %	Oh	0.1487	-0.4183	-0.0754	-0.4006	0.1612	0.7170	0.0592
Clay %	Bh	0.0888	-0.3594	-0.3501	-0.2664	-0.4594	0.0515	-0.5194
Silt %	Bh	0.1686	0.0572	0.1730	0.5220	-0.0857	-0.1489	0.1767
Sand %	Bh	-0.1728	0.1883	0.1051	-0.1968	0.3709	0.0729	0.2208
pH	Oh	-0.3584	0.1345	-0.2052	0.0720	0.1256	-0.3179	-0.0434
	Eh	-0.0558	0.4722	0.4694	0.5664	0.3402	-0.5762	0.4300
	Bh	-0.2716	0.2821	0.3138	0.2415	0.4504	-0.6137	0.0790
Ca_{Al} mg/kg	Oh	-0.0568	-0.1903	-0.0022	-0.1498	0.0521	0.4254	0.3674
	Eh	0.0190	-0.3356	-0.4038	-0.4207	-0.4188	0.0878	-0.5444
	Bh	0.2948	0.3524	0.0753	-0.3463	-0.2737	-0.1476	-0.1790
K_{Al} mg/kg	Oh	0.0726	-0.2351	-0.1217	-0.3077	-0.1487	0.5909	0.1907
	Eh	0.0340	-0.3359	-0.4355	-0.4712	-0.4385	0.1977	-0.5182
	Bh	0.2497	0.3161	-0.0366	-0.3340	-0.3164	-0.1784	-0.5050
Mg_{Al} mg/kg	Oh	0.2238	0.4758	0.3262	-0.1629	0.0190	0.0148	0.0485
	Eh	-0.1519	0.1522	-0.2406	-0.2193	-0.3089	-0.2179	-0.3256
	Bh	0.2988	0.4621	0.0870	-0.3746	-0.2330	-0.1308	-0.2941
Na_{Al} mg/kg	Oh	0.3152	0.4151	0.1431	-0.3238	-0.1378	-0.0637	-0.2389
	Eh	-0.1600	0.2020	0.3644	0.1572	0.6043	-0.2165	0.3687
	Bh	0.3137	0.4583	0.0840	-0.3865	-0.2430	-0.0944	-0.2690
P_{Al} mg/kg	Oh	0.2905	-0.0386	-0.1152	-0.5536	-0.3341	0.4401	-0.1869
	Eh	0.0332	-0.3825	-0.4192	-0.4579	-0.4126	0.1443	-0.5495
	Bh	0.1947	0.1154	-0.1576	-0.3113	-0.3447	-0.1077	-0.5888
P_{ox} mg/kg	Eh	0.0335	-0.3758	-0.4243	-0.4652	-0.4169	0.1597	-0.5436
	Bh	-0.0118	-0.2024	-0.4086	-0.4233	-0.4409	0.1115	-0.4830
Fe_{ox} mg/kg	Eh	-0.1113	-0.0358	-0.1804	-0.0938	-0.2629	-0.4291	-0.4441
	Bh	-0.0696	0.4771	0.3976	0.6971	0.2083	-0.6047	0.3603
Al_{ox} mg/kg	Eh	0.0244	-0.4457	-0.4086	-0.4874	-0.3592	0.1240	-0.5776
	Bh	0.0081	0.3126	0.3142	0.6748	0.0865	-0.5401	0.2496

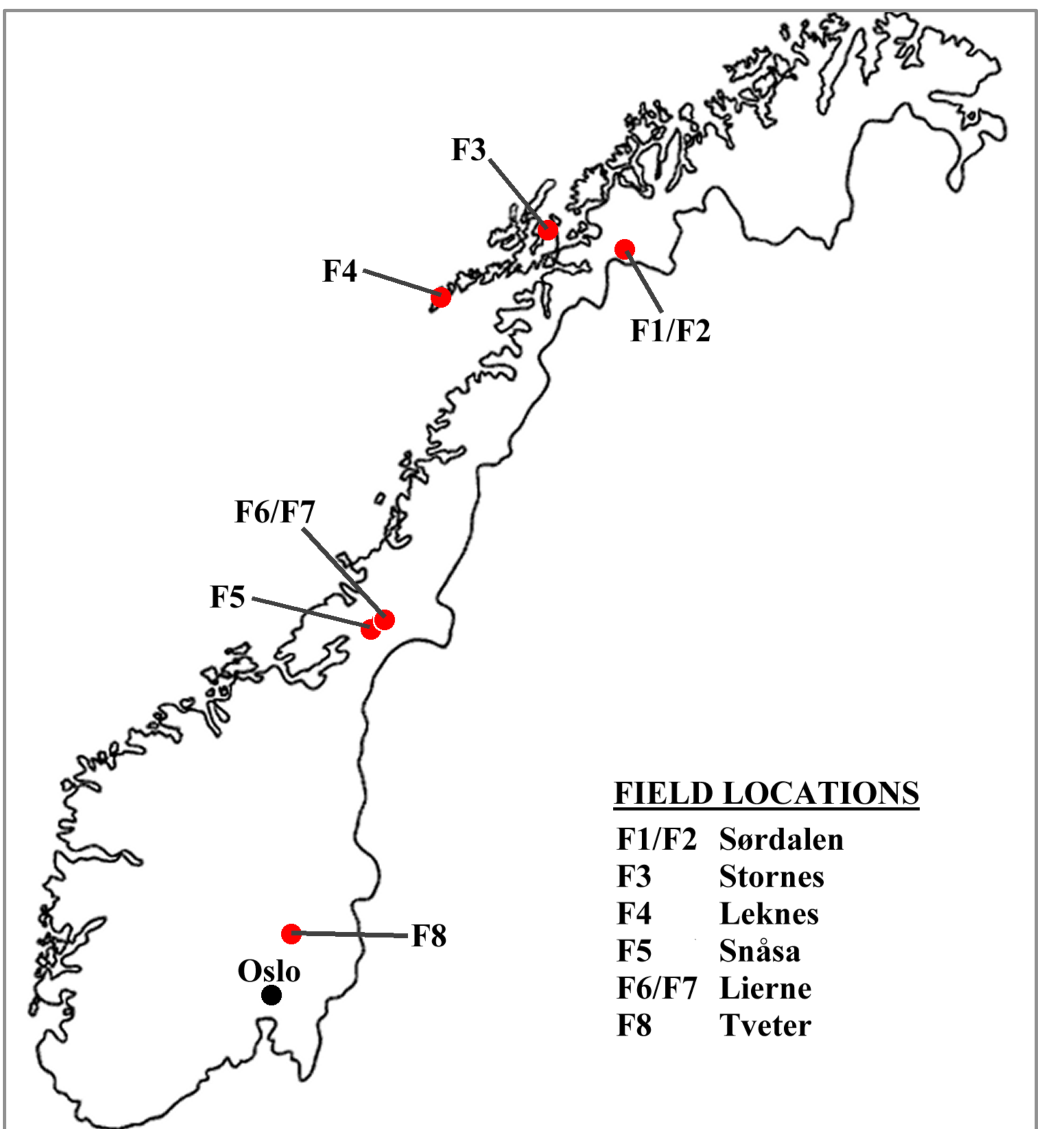
Oh = O-layer
Eh = E-layer
Bh = B-layer
$_{Al}$ = ammonium lactate extractable
$_{ox}$ = oxalic acid extractable

Supplementary Tab. S3: Pearson correlation between quality parameters of berry samples based on replicate data from single measurements (all field trials).

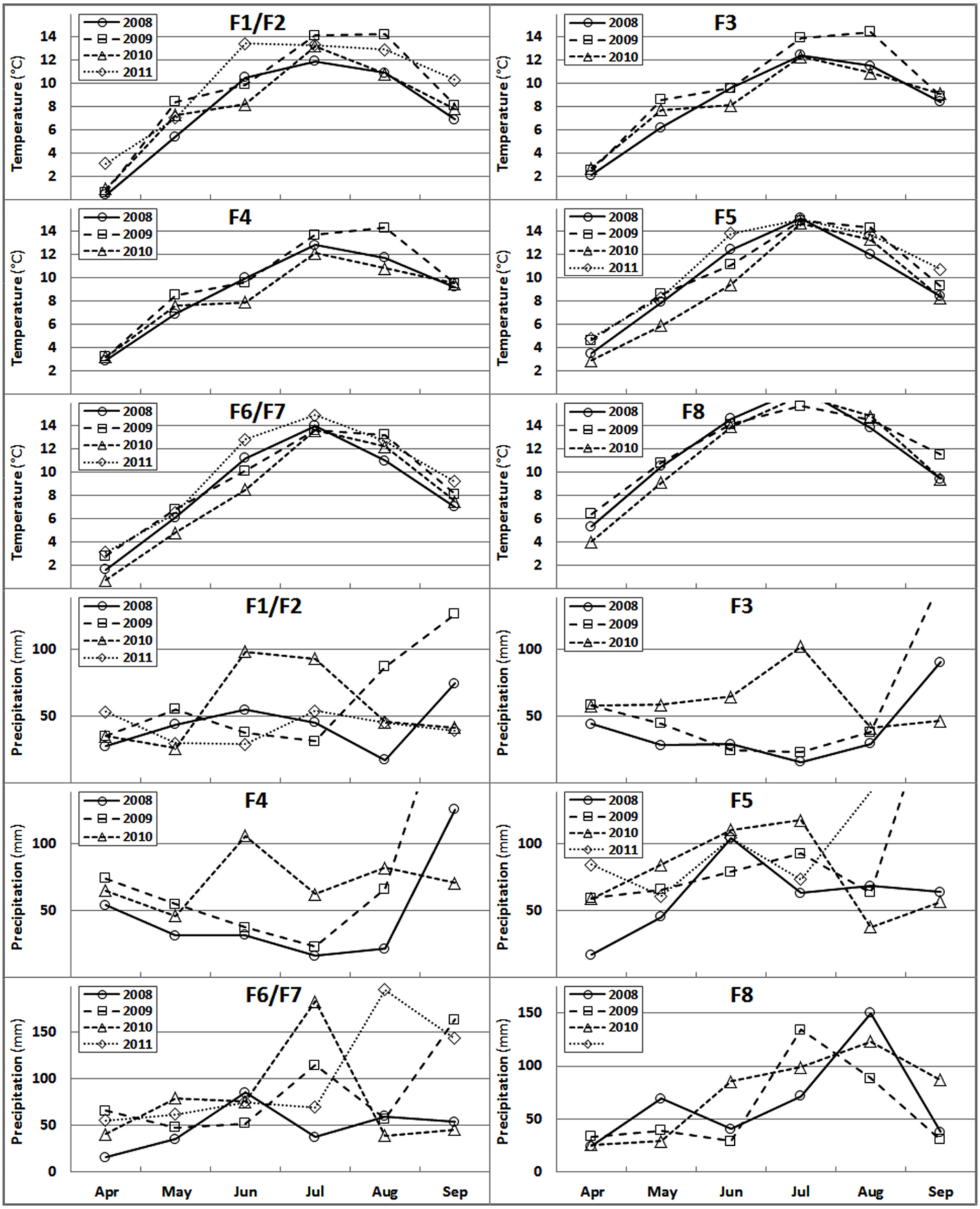
	ACY	TPH	AOX	SS	TA	pH	DM
TPH	0.125						
AOX	0.170	0.497					
SS	-0.339	0.391	0.268				
TA	-0.298	0.250	0.168	0.450			
pH	0.357	-0.151	-0.036	-0.414	-0.537		
DM	-0.142	0.267	0.298	0.551	0.274	-0.114	
Berry weight	-0.174	-0.402	-0.409	-0.198	-0.252	0.092	-0.674

Supplementary Tab. S4: Pearson correlation between quality parameters of berry samples based on replicate data from single measurements at different harvest time points at trial field F8 (Tveter).

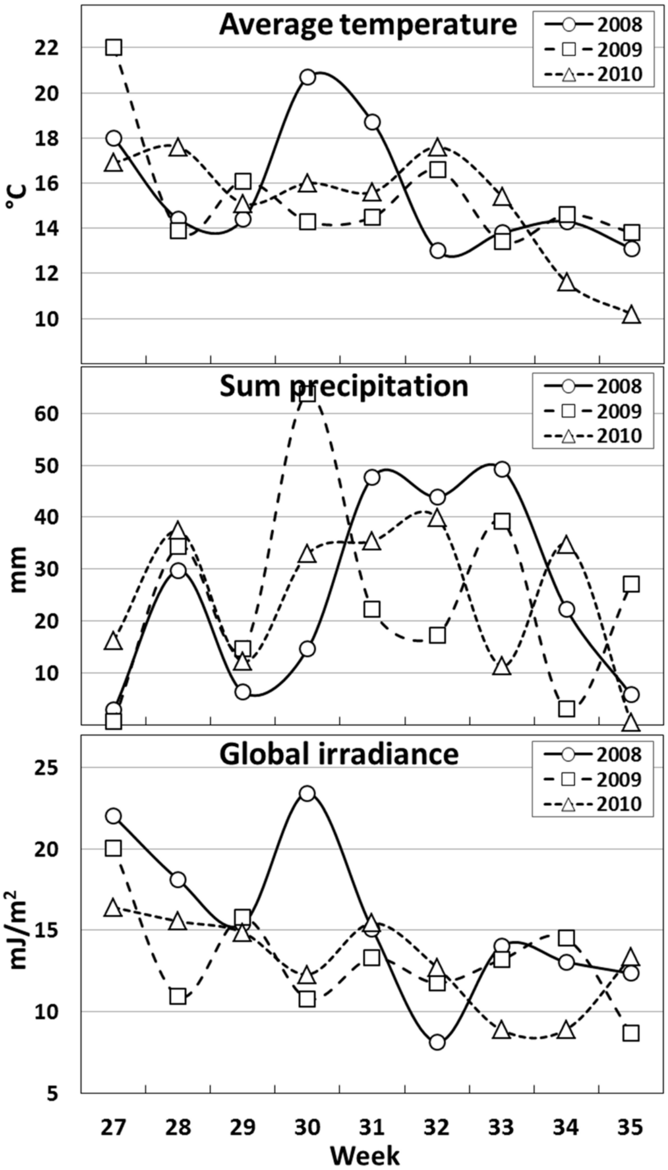
	ACY	TPH	AOX	SS	TA	pH	DM
TPH	-0.028						
AOX	0.068	0.535					
SS	-0.543	0.300	0.038				
TA	-0.604	0.086	-0.284	0.518			
pH	0.431	0.000	0.129	-0.518	-0.602		
DM	-0.400	0.265	-0.027	0.853	0.512	-0.435	
Berry weight	0.172	0.123	0.327	-0.720	-0.380	0.594	-0.831



Supplementary Fig. S1: Trial field locations in Norway.



Supplementary Fig. S2: Monthly average temperature and precipitation data for all field locations throughout the growing season (April to September) from 2008 to 2011. F1/F2 (Sørdalen), F3 (Stornes), F4 (Leknes), F5 (Snåsa), F6/F7 (Lierne) and F8 (Tveter).



Supplementary Fig. S3: Weekly average temperature, precipitation and global irradiance for field F8 (Tveter) in Southern Norway throughout the summer season in July and August (weeks 27 to 35) from 2008 to 2010.