Influence of genotype and climatic environment on fruit yield and chemical composition of black currant (*Ribes nigrum* L.)

Virkning av genotype og klima på avling og kjemisk innhold hos solbær (*Ribes nigrum* L.)

Philosophiae Doctor (PhD) Thesis

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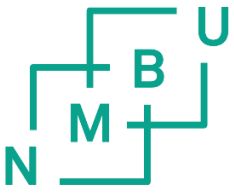

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Ås, March 2016

Tomasz Woznicki

Summary

Black currant (*Ribes nigrum* L.) is a widely cultivated soft fruit species, which is renowned for its high berry concentrations of proven, or presumed, health-promoting compounds.

Over the last years, there has been a growing interest in the effects of genotype and environmental conditions on the quality of fruits and berries including the black currant. In the present study, we have examined the impact of environmental conditions on yield and chemical composition of seven black currant cultivars from different national breeding programs. Two experimental approaches were used: 1) Correlation analyses of the relationship between berry chemical content and weather conditions in the field at Ås, Norway (59°40'N), over a period of eight years (Paper I), and 2) Studies on the effects of post-flowering temperature and photoperiod on berry chemical content in controlled (phytotron) environments. In addition, plants of all cultivars were also grown outdoors under ambient conditions as a control (Paper II, III, IV).

Black currant berry yield and weight were negatively correlated with summer temperature and positively correlated with precipitation. Elevated ripening temperature increased dry matter as well as soluble solid contents and pH of the berries. In addition, black currants had higher content of ascorbic acid, total monomeric anthocyanins and total phenolic compounds in years with cool summers with ample precipitation. In general, years with relatively low temperature and ample precipitation enhanced yield and increased the nutritional quality of the berries.

Cultivation of single-stemmed potted plants of four black currant cultivars in a phytotron at constant temperatures of 12, 18 or 24 °C and different photoperiods (short day, short day with night interruption, and natural summer daylight conditions) generally supported the results from the field experiment. Thus, accumulation of both forms of ascorbic acid [L-ascorbic acid (AA) and dehydroascorbic acid (DHAA)] decreased with increasing ripening temperature over the 12-24 °C range, while the ratio between AA and DHAA increased. Likewise, the concentration of hexose sugars and, to lesser extent sucrose, decreased with increasing temperature, whereas the concentration of citric acid, which is the predominant organic acid in black currant berries, increased. This resulted in an increased sugar/acid-ratio in berries ripened under low temperature conditions. The concentration of total monomeric anthocyanins in the berries was highest at 18 °C, with both higher and lower temperatures resulting in lower accumulation. Total phenolic concentration in berries ripened under controlled climate conditions remained relatively stable

across the different temperature and light regimes, whereas the antioxidant capacity was reduced at high temperature.

The predominating anthocyanins in the studied black currant cultivars were delphinidin-3-rutionside and cyanidin-3-rutinoside, which accounted for 75-83 % of the total under the various environmental conditions. Analysis of individual anthocyanins, flavonols and hydroxycinnamic acids revealed different, and sometimes, opposite patterns of accumulation for compounds in the same subclass across the temperature regimes. Increased temperature over the 12-24 °C range caused a significant increase in the concentrations of delphinidin-3-glucoside, delphinidin-3-(6”-coumaroyl)-glucoside and cyanidin-3-(6”-coumaroyl)-glucoside, while the opposite trend was observed for cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside. The highest accumulation of delphinidin-3-rutinoside was observed at 18 °C.

Daylength had only minor impacts on accumulation of health related phytochemicals. In general, there were no significant differences between berries ripened under different photoperiodic treatments with identical daily light energy. On the other hand, increased daily light integral under natural daylength conditions in the phytotron stimulated the accumulation of total monomeric anthocyanins, and he same response was observed under increased radiation in the field.

The concentrations of all anthocyanins and some flavonols were higher in berries ripened outdoors than in the phytotron, apparently due to screening of UV-B radiation by the glass cover.

In general, plant genetic background was the main source of variation in fruit quality and had supreme influence on accumulation of bioactive compounds in black currant berries. Under both field and phytotron conditions, significant cultivar differences in berry chemical composition were observed. The present investigation also revealed that black currant cultivars may vary significantly in their inherent stability in accumulation of the various chemical compounds across varying environmental conditions.

In conclusion, the present results demonstrate that plant genotype and climatic conditions during the ripening period had strong influence on chemical composition of black currant berries. The presented results may contribute to a deeper understanding of the complex relations between climate environmental conditions and berry nutritional quality of black currants.

Sammendrag

Solbær (*Ribes nigrum* L.) er kjent for sine høye konsentrasjoner av en rekke helsefremmende stoffer. I Norge er solbær mest dyrket til industri, men noe dyrkes også til friskkonsum. Den er vanlig i privathager, og interessen for arten er økende.

I den senere tid har det vært en økende interesse for å undersøke effekten av genotype og klima på helserelatert kvalitet av frukt og bær. Formålet med dette arbeidet, var å undersøke virkningen av ulike klima-forhold på avling og kjemisk innhold i syv solbærsorter av ulik genetisk opprinnelse. I forsøkene ble det brukt to ulike fremgangsmåter: 1) Korrelasjon mellom ulike kjemiske innholdsstoffer i bær og klimaforhold i Ås, Norge (59°40'N) over en periode på åtte år (Paper I), og 2) virkninger av temperatur og daglengde på kjemisk innhold i bær under kontrollerte betingelser (fytotron). I tillegg ble alle sortene dyrket utendørs under naturlige betingelser som kontroll (Paper II, III, IV).

I felt var avling og bærvekt negativt korrelert med sommertemperatur, men positivt korrelert med nedbør. Tørrstoffinnhold, så vel som oppløst tørrstoff og pH, økte med økende temperatur. Derimot hadde bæra høyere innhold av askorbinsyre, totale monomere anthocyaniner og totale fenoler i år med kjølige somre og rikelig med nedbør. Generelt økte avlingen og den ernæringsmessige kvaliteten i år med relativt lav sommertemperatur og rikelig med nedbør.

Resultatene fra dyrking av en-stammete planter av fire solbærsorter i fytotron ved konstante temperaturer (12, 18 eller 24 °C) og ulik daglengde (kort dag, kort dag med nattavbrudd, og naturlige dagslys-forhold), støttet opp om resultatene fra feltforsøket. Innhold av begge formene for askorbinsyre [L-askorbinsyre (AA) og dehydroaskorbinsyre (DHAA)] avtok med stigene modningstemperatur i området 12-24 °C, mens forholdet mellom AA og DHAA økte. På tilsvarende måte avtok konsentrasjonen av glukose og fruktose, og i mindre grad, konsentrasjon av sukrose i bærene ved økende temperatur. Omvendt effekt ble funnet for sitronsyre, som er den dominerende organiske syren i solbær, hvor konsentrasjonen økte ved høyere modningstemperatur. Dette resulterte i et økt sukker/syre-forhold i bær modnet ved lave temperaturer. Konsentrasjonen av monomere anthocyaniner var høyest ved 18 °C, mens både høyere og lavere temperaturer førte til lavere akkumulering. Konsentrasjonen av totale fenoler i bær modnet under kontrollerte betingelser holdt seg relativt stabil, mens antioksidantkapasiteten gikk ned ved økende modningstemperatur.

Dominerende anthocyaniner i de ulike solbærsortene var delphinidin-3-rutionside og cyanidin-3-rutinoside, som utgjorde 75-83 % av det totale anthocyanin-innholdet. Både for anthocyaniner, flavonoler og hydroxykanel-syrer, ble det funnet ulike og til dels motsatte mønstre for akkumulering under ulike temperaturforhold. Konsentrasjonen av delphinidin-3-glukosid, delphinidin-3-(6"-coumaroyl)-glukosid og cyanidin-3-(6"-coumaroyl)-glukosid økte markant med økende modningstemperatur, mens en motsatt trend ble observert for cyanidin-3-glukosid, cyanidin-3-rutinosid, og peonidin-3-rutinosid. Den høyeste konsentrasjonen av delphinidin-3-rutinoside ble funnet i bær modnet ved 18 °C.

Daglengden hadde liten påvirkning på innholdet av helserelaterte inholdsstoffer i solbær. Generelt ble det ikke funnet signifikante forskjeller i solbær modnet ved ulike daglengder, når den daglige lysenergien ble holdt konstant. Derimot førte økt lysenergi under naturlige daglengde-forhold i fytotron til økt akkumulering av totale monomere anthocyaniner, og den samme trenden ble observert ved økene lysstråling i felt. Innhold av totale anthocyaniner og flavonoler var høyere i bær modnet utendørs enn i fytotron, trolig på grunn av filtrering av UV-B-stråling i glasstaket i fytotronen.

Genetisk sammensetning var den viktigste årsaken til variasjon i fruktkvaliteten og var av overordnet betydning for akkumulering av bioaktive forbindelser i solbær. Det ble observert betydelige forskjeller i den kjemiske sammensetningen mellom de ulike solbærsortene både i felt og fytotron. De ulike solbærsortene varierte også betydelig i stabilitet for akkumulering av ulike kjemiske forbindelser under varierende miljøforhold.

Resultatene i dette arbeidet viser at genotype og klima-forhold under modningsperioden hadde sterk innflytelse på innhold av kjemiske forbindelser i solbær. Disse resultatene kan bidra til en dypere forståelse av den komplekse sammenhengen mellom klima/miljø-forhold og den ernæringsmessige kvaliteten av solbær.

List of papers

This thesis is based on the following papers referred to in the text by their Roman numerals:

I. Woznicki, T. L., Heide, O. M., Sønsteby, A., Wold, A.-B. & Remberg, S. F. (2015). **Yield and fruit quality of black currant (*Ribes nigrum* L.) are favoured by precipitation and cool summer conditions.** *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, *65*: 702-712

II. Woznicki, T. L., Heide, O. M., Sønsteby, A., Wold, A.-B. & Remberg, S. F. (2015). **Effects of controlled post-flowering temperature and daylength on chemical composition of four black currant (*Ribes nigrum* L.) cultivars of contrasting origin.** *Scientia Horticulturae*, 197: 627-636

III. Woznicki, T. L., Sønsteby, A., Aaby, K., Martinsen, B. K., Heide, O. M., Wold, A.-B. & Remberg, S. F.
Ascorbate pool, sugars and organic acids in black currant (*Ribes nigrum* L.) berries are strongly influenced by genotype and post-flowering temperature. *Journal of the Science of Food and Agriculture*, submitted

IV. Woznicki, T. L., Aaby, K., Sønsteby, A., Heide, O. M., Wold, A.-B. & Remberg, S. F. (2016). **Influence of controlled post-flowering temperature and daylength on individual phenolic compounds in four black currant cultivars.** *Journal of Agricultural and Food Chemistry*, 64: 752-761

1. General introduction

1.1 Origin, taxonomy and biology

Black currant (*Ribes nigrum* L.) is indigenous to central and northern Europe, Caucasus, Central Siberia and Himalaya. It is a woody shrub growing up to 2 m in height with smooth, alternate leaves, up to 10 cm of length. All parts of the plant have a strong, specific aroma. The flowers are produced in racemes (known as "strigs") up to 8 cm long, with ten to twenty flowers, each about 8 mm in diameter. The flowers have five hairy sepals, which are longer than petals. There are five stamens surrounding the stigma and style and two fused carpels. The flowers are mostly insect pollinated, but some pollen is distributed by the wind. The berries are shiny black when fully ripe and up to 10 mm in diameter (Hummer and Barney, 2002). Black currant is diploid (2x = 2n = 16), and natural polyploids are rare) (Brennan, 2008).

Domestication of black currant has taken place only within the last 400 years (Brennan, 1996), however, it is known, that plants were cultivated already in the 11th century in Russian monasteries (Doronina and Terekhina, 2009).

Black currants were first imported to the UK from Holland by Tradescant in 1611 and these are probably the plants that were first described in John Gerard's book *The Herball or General Historie of Plantes* (2nd edn.,1636) (**Figure 1**). The European black currant was introduced in North America around 1629 (Brennan, 1996). In Norway, black currants were described for the first time in 1743 (Langeland, 2008).

Black currant belongs to the genus *Ribes*, containing about 150 spiny and non-spiny species of shrubs (Brennan, 2008) with the botanical classification shown in **Table 1**.

The commonly used edible species of *Ribes* are the black currants (*Ribes nigrum* L.), red and white currants (*R. rubrum* L., synonyms = *R. vulgare* Jancz. and *R. sativum* Syme.) as well as gooseberry (*Ribes uva-crispa* L., synonym = *R. grossularia* L., and American gooseberry *Ribes hirtellum* Michx.)

Table 1. Taxonomy of the *Ribes* genus (USDA, 2016)

Kingdom: *Plantae* – Plants
Subkingdom: *Tracheobionta* – Vascular plants
Superdivision: *Spermatophyta* – Seed plants
Division: *Magnoliophyta* – Flowering plants
Class: *Magnoliopsida* – Dicotyledons
Subclass: *Rosidae*
Order: *Rosales*
Family: *Grossulariaceae* – Currant family
Genus: *Ribes* L. – currant

3 Beſides theſe there is another, which differs little from the former in ſhape, yet grows ſomewhat higher, and hath leſſer leaues: the floures are of a purpliſh greene colour, and are ſucceeded by fruit as big againe as the ordinary red, but of a ſtinking and ſomewhat loathing fauour: the leaues alſo are not without this ſtinking ſmell.

¶ *The Place, Time, and Names.*

None of theſe grow wild with vs, but they are to be found plentifully growing in many gardens, eſpecially the two former, the red and the white.

The leaues and floures come forth in the Spring, and the fruit is ripe about Midſommer.

This plant is thought to haue been vnknowne to the Antient Greekes: ſome thinke it the *Ribes* of the Arabian *Serapio*. *Fuchſius*, *Matthiolus*, and ſome other deny it; notwithſtanding *Dodonæus* affirmes it: neither is the controuerſie eaſie to be decided, becauſe the Author is brieſe in the deſcription thereof, neither haue we his words but by the hand of a barbarous Tranſlator. However the ſhops of late time take it (the faculties conſenting thereto) for the true Ribes, and of the fruit hereof prepare their *Rob de Ribes*. *Dodonæus* calls it *Ribeſium*, *groſſularia rubra*, & *Groſſularia tranſmarina*; and they are diſtinguiſhed into three ſorts, *Rubra*, *Alba*, *Nigra Ribeſia*, red, white and blacke Currans: the Germans call them **S. Johans traubell**, or **traublin**, and **S. Johans Beerlen**: the Dutch, **Beſſkins ouer Zee**: the Italians, *Vuetta roſſa*: the French, *Groiſſeles*, *Groſeilles d'outre mer*: the Bohemians, **Jahodi S. Jana**: the Engliſh, Red Currans: yet muſt they not be confounded

with

Figure 1. The early description of *Ribes.* In: Gerard, J. (1636) The Herball or General Historie of Plantes (2nd ed.), London: Printed for Adam Islip, Joice Norton, and Richard Whitakers.

1.2 Cultivation

Black currant plants are usually propagated by hardwood cuttings in autumn, softwood cuttings in spring or single bud cuttings. The most common practise is to take 15-25 cm long cuttings in the fall from dormant bushes and plant them directly after cutting or in the spring after overwintering in a cold store. Seed propagation is mainly used in breeding programs and requires seed stratification at 2-4 ^{o}C for 12-14 weeks, and a germination temperature at about 16-20 ^{o}C.

Modern black currant plantations have a density of approximately 5 000 plants per hectare, with 0.4-0.5 m between plants in the row, and 3.8-4.2 m between rows to allow mechanical harvesting. Commercially, the main harvesting method is by the use of straddle harvesters. Average fruit yield is approx. 7 tons/ha. Application of 100 kg N, 20 kg P, 40 kg K per hectare per year is commonly practiced (Harmat et al., 1990). Most growers prune the plants by taking out branches growing outwards from the row, in addition to damaged and old branches. After 5-10 years, the plantation may be cut to ground level to regenerate.

Most of the commercial black currant production is located to central, eastern and northern Europe. According to FAO (2013), the biggest global producer of currant fruits is the Russian Federation (346 000 tons), followed by Poland (194 522 tons), Ukraine (24 100 tons), Austria (19 140 tons) UK (15 400 tons) and Scandinavia (13 835 tons). However, China is also a great contributor, but statistics is not provided by FAO. Black currant production in North America is marginal because of prohibition of cultivation of *Ribes* species, due to white pine blister rust (*Cronartium ribicola*) for which *Ribes* species are alternate hosts. Recently, some states retreat this law and there is currently a growing interest for black currant production in USA and Canada.

At present, the most important threats to black currant production are pest and disease. Gall mite (*Cecidophyopsis ribis* Westw.) can be most dangerous for black currant, mainly due to its role as a vector of black currant reversion virus (BRV). This causes sterility of the plants within two years. In recent years, occurrence of mites increased due to the ban of many pesticides. However, there are known sources of resistance to both gall mite and the virus: the Ce genes from gooseberry (Knight et al., 1974) and P from *Ribes nigrum* var. *sibiricum* (Anderson, 1971). BRV resistance from *Ribes dikuscha* is used in the cultivars ‘Golubka’ and ‘Ben Gairn’, although the genetic control of resistance is not known (Brennan and Jarret, 2014). Other major pests of *Ribes* are leaf-curling midge (*Dasineura tetensii* Rübs.), sawfly (*Nematus ribesii*) and, especially in New

Zealand, currant clearwing (*Synanthedon tipuliformis*). The most damaging species of aphids are *Hyperomyzus lactucae*, *Cryptomyzus galeopsidis*, *Cryptomyzus ribis* and *Aphis schneideri*.

Important foliar diseases on blackcurrant include mildew (*Sphaerotheca mors-uvae*), leafspot (*Drepanopeziza ribis*), botrytis (*Botrytis cinerea*), white pine blister rust (*Cronartium ribicola*) and septoria leafspot (*Septoria ribis*). For most of these diseases resistance genes are known (Brennan, 2008). The main mildew resistant cultivars are the Scottish 'Ben Hope', 'Ben Gairn', 'Ben Dorain', the Polish cultivars 'Tiben' and 'Tisel' and the Russian `Pilot Alexander Mamkin'. The Canadian cultivars 'Consort', 'Coronet', 'Crusader' and the Polish 'Tihope' are rust resistant (Brennan, 2006; Pluta and Żurawicz, 2015).

A genetic linkage map of black currant evaluating important fruit quality and phenological traits have been constructed. In addition, quantitative trait loci (QTLs) affecting these properties are associated in the linkage map (Brennan et al., 2008).

Another serious threat to the future of black currant production is insufficient winter chill during the winter period in the wake on the ongoing global climatic change. There is now a growing concern about the negative effects this may have on flowering, bud brake and spring frost damage in areas with mild winter climate (Hedley et al., 2010; Sønsteby and Heide, 2014).

1.3 Chemical composition

Black currant berries are an excellent source of many health related phytochemicals such as phenolic compounds and vitamin C as well as organic acids and sugars (Brennan and Graham, 2009).

According to Heiberg et al. (1992) black currant berries contain approximately 15 % of soluble solids and 9 % of sugars (fructose 45 %, glucose 40 % and sucrose 15 %) on a fresh weight basis as an average for ten cultivars. The relatively low sucrose concentration in berries may be a result of enzymatic hydrolysis of sucrose to glucose after translocation from the leaves (Forney and Breen, 1985). The acid content was 5 % of the fresh weight, with > 80 % citric acid and approx.10 % malic acid. Black currants also contain other organic acids such as quinic and shikimic acid. Citric acid is the main organic acid responsible for berry acidity (Rubinskiene et al., 2006). The balance between sugars and organic acids differ between cultivars and is important for the sensory characteristics of the berries (Kaldmäe et al., 2013).

Black currants are known for their relatively high concentration of vitamin C, which belongs to the group of water-soluble vitamins, and this term (vitamin C) is used for all compounds with similar biological activity. There are two forms known; ascorbic acid (AA) and dehydroascorbic acid (DHAA), reduced and oxidized, respectively, and both forms contribute to vitamin C activity (**Figure 2**). The total ascorbic acid pool in black currant includes approx. 20 % of DHAA. Vitamin C in black currant berries is produced *in situ* via the L-galactose pathway, consisting of transformation of D-glucose to GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose and L-galactono-1,4-lactone (Hancock et al., 2007). The ascorbic acid structure is characterized as an aldono-1,4-lactone of hexonic acid (Davey et al., 2000).

Concentration of vitamin C varies greatly between cultivars, from less than 70 mg/100 g to more than 350 mg/100 g of fresh weight (Brennan and Graham, 2009; Nes et al., 2012; Vagiri et al., 2013). According to Levine et al. (1996) the recommended daily human allowance should be as high as 200 mg of vitamin C.

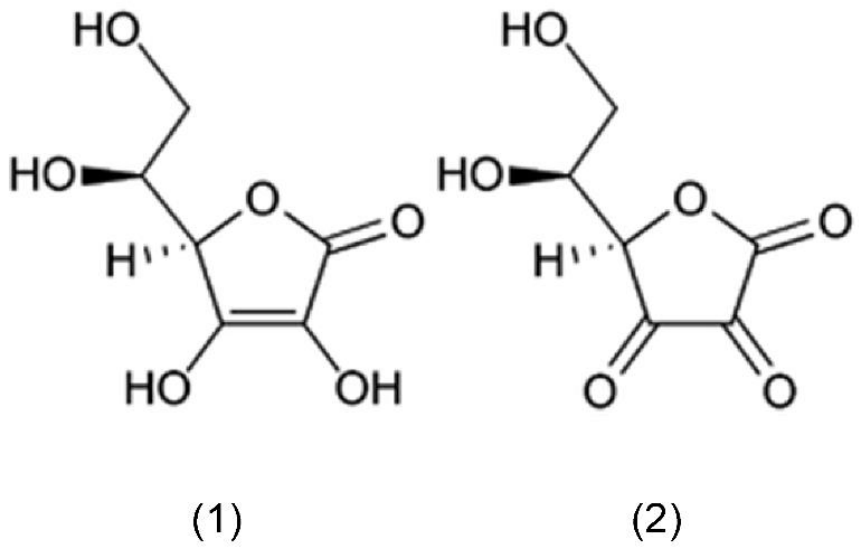


Figure 2. Structures of (1) ascorbic acid and (2) dehydroascorbic acid.

Phenolic compounds are widely distributed secondary plant metabolites responsible for the majority of the sensory and health promoting properties of fruits and medicinal plants. Classification of phenolic compounds is based mainly on the number of phenol rings (phenolic acids, stilbenes, flavonoids, lignans and tannins). The flavonoids are the main bioactive compounds found in fruits (Haminiuk et al., 2012; Del Rio et al., 2013).

Biosynthesis of phenolic compounds is located in the cytosol of plant cells (Jaakola and Hohtola, 2010), originating from acetyl-coenzyme A and C6•C3 precursors (p-coumaric, ferulic, sinapic and caffeic acids). Often, the precursors are called the "hydroxycinnamate pool" and are derived from α-amino acids (L-phenylalanine and/or L-tyrosine) (Zheng et al., 2012). The general phenylpropanoid pathway starts with the conversion of phenylalanine to cinnamic acid catalyzed by phenylalanine ammonia lyase (PAL) (**Figure 3**). The following product is *p*-coumaric acid, a precursor of hydroxycinnamic acid conjugates. Chalcone synthase (CHS) provides the condensation of *p*-coumaroyl-CoA (the next product from the general phenylpropanoid pathway) with three molecules of malonyl-CoA to produce naringenin chalcone. This is the beginning of the pathway leading to the production of numerous flavonoids (Jaakola and Hohtola, 2010). Flavonoids can be divided into six main subclasses including: flavonols, flavonones, isoflavones, flavan-3-ols, flavones and anthocyanidins (Ververidis et al., 2007; Haminiuk et al., 2012) and have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and one heterocyclic ring (C) (**Figure 4**). After biosynthesis, flavonoids are transported to vacuoles or cell walls where they are present mainly as glycosides (Jaakola and Hohtola, 2010).

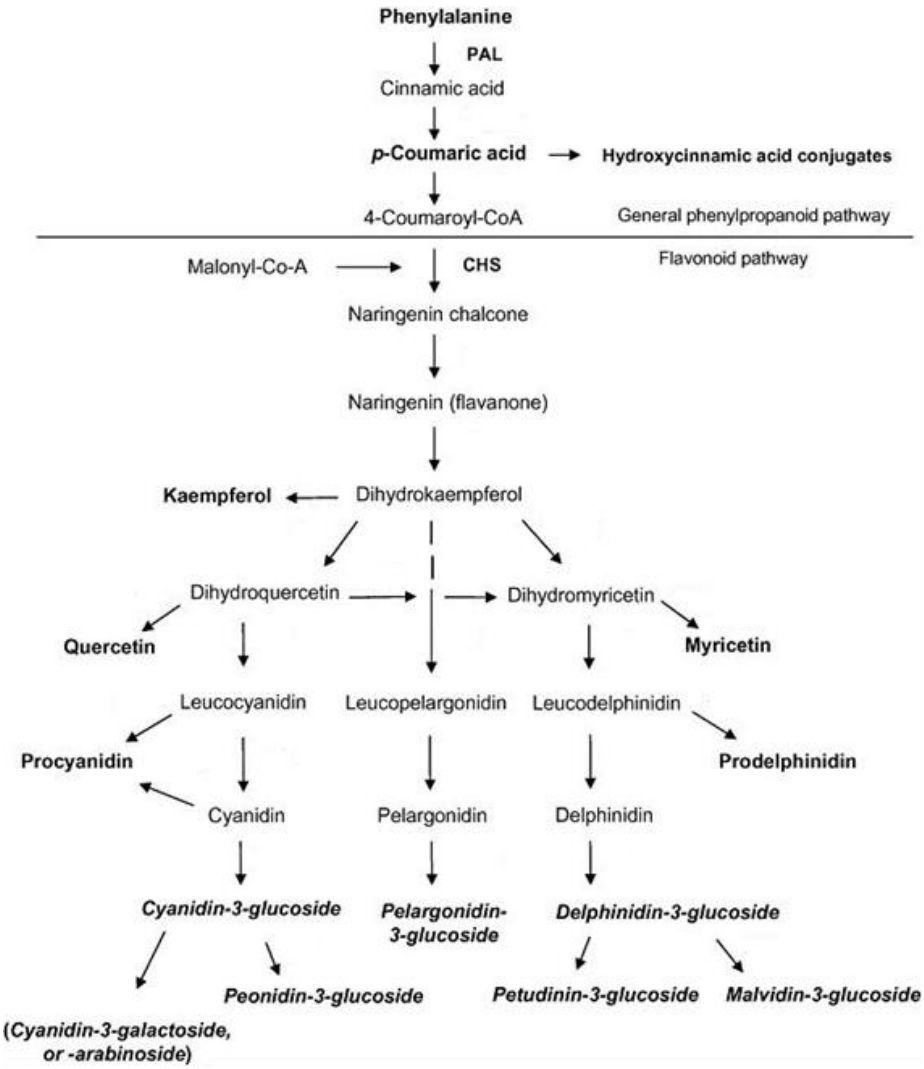


Figure 3. The general phenylpropanoid and flavonoid pathways. Key enzymes: PAL - phenylalanine ammonia lyase, CHS - chalcone synthase (adapted from: Jaakola and Hohtola, 2010).

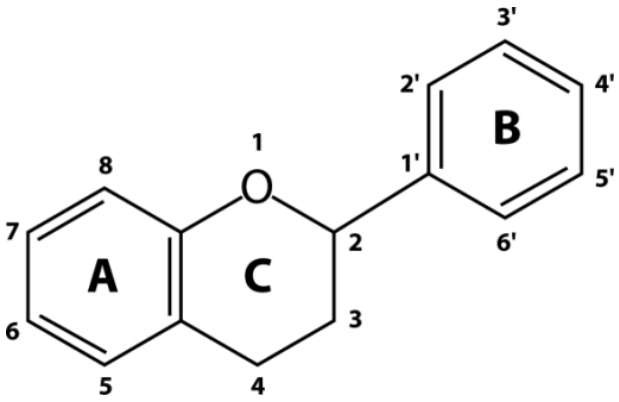


Figure 4. Basic structure of a flavonoid (adapted from: Jaakola and Hohtola, 2010).

Anthocyanins, which are glycosides of anthocyanidins (**Figure 5**), are important pigments in leaves, flowers and fruits, responsible for red, purple and blue colours. They also serve as a protecting agents as well as attractants for pollinators and seed dispersers (Koes et al., 2005). The stability of anthocyanins is pH dependent. At low pH the colour of anthocyanins is stable, whereas at higher pH the colourless chalcone forms are present. Black currants may contain up to fifteen anthocyanin structures, including delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, petunidin 3-O-glucoside, petunidin 3-O-rutinoside, cyanidin 3-O-arabinoside, pelargonidin 3-O-glucoside, pelargonidin 3-O-rutinoside, peonidin 3-O-glucoside, peonidin 3-O-rutinoside, malvidin 3-O-glucoside, malvidin 3-O-rutinoside, delphinidin 3-O-(6‘‘coumaroylglucoside) and cyanidin 3-O-(6‘‘-coumaroylglucoside). However, only four compounds contribute to more than 90 % of the total anthocyanin content in black currant (3-O-glucosides and the 3-O-rutinosides of delphinidin and cyanidin) (Slimestad and Solheim, 2002).

Analyses of anthocyanin profiles in 33 black currant cultivars revealed that for 26 cultivars delphinidin 3-O-rutinoside was the predominant anthocyanin, whereas 6 cultivars had the highest concentration of cyanidin 3-O-rutinoside and only one had delphinidin 3-O-glucoside as the most abundant anthocyanin (Hellström et al., 2010). The authors observed a twofold difference in total anthocyanin content between cultivars with the highest, respective lowest content.

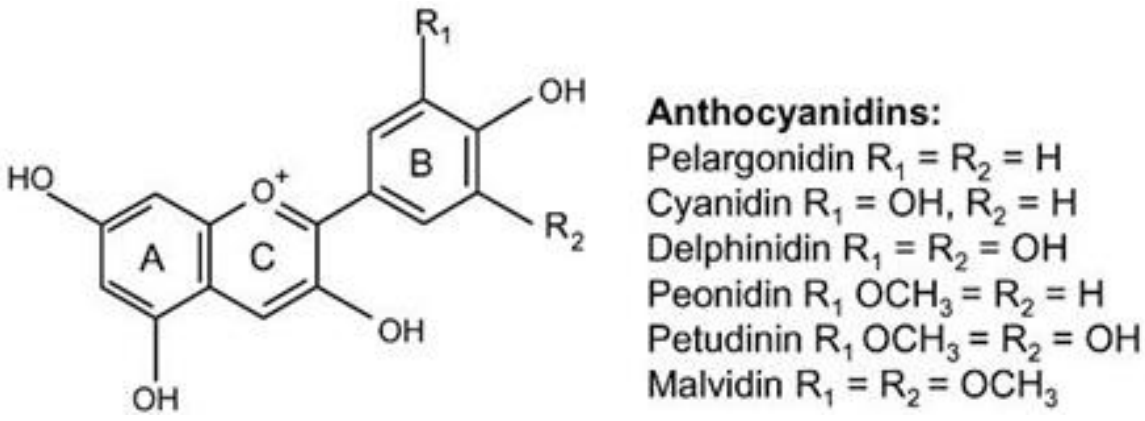


Figure 5. Structures of anthocyanidins (adapted from: Jaakola and Hohtola, 2010).

Flavonols are a subclass of flavonoids that have the 3-hydroxyflavone backbone. Their diversity is based on different positions of the phenolic -OH groups on the B-ring (**Figure 4**). The detailed differences in flavonol structures are presented in **Figure 6**. Black currant contains glycosylated forms of flavonols, mainly myricetin, quercetin and kaempferol. Glycosylation (linkage of the sugar to the 3-hydroxyl group), increases flavonol polarity enabling storage of these compounds in the vacuoles (Aherne and O'Brien, 2002). Flavonols can influence plant responses to stress conditions such as low temperature, excess light, drought and toxins (Treutter, 2006).

Results obtained by Mikkonen et al. (2001) demonstrate relatively wide variations in the major flavonol content among 10 black currant cultivars. For all black currant cultivars, myricetin was the predominant flavonol, followed by quercetin and kaempferol. The content of flavonols (myricetin, quercetin and kaempferol) varied from 17.9 mg/100 g FW to 38.3 mg/100 g FW.

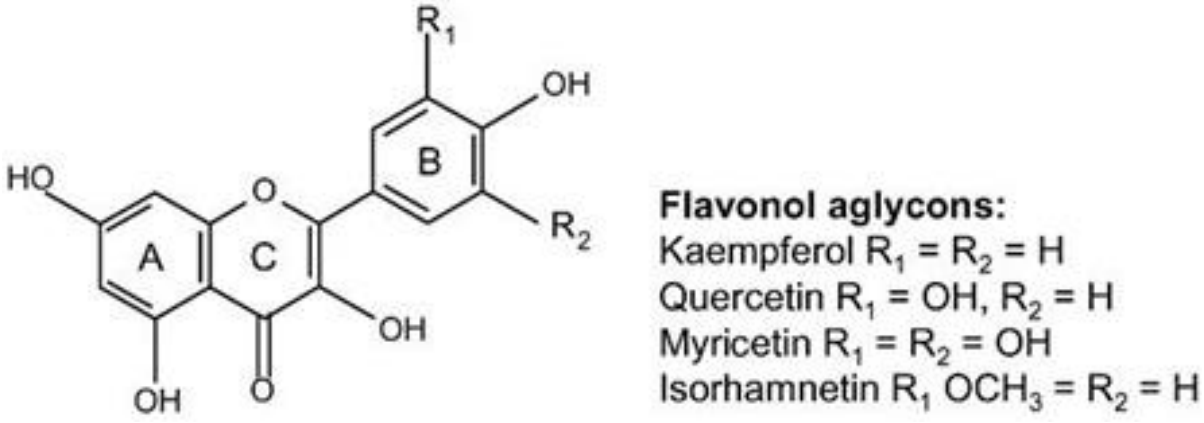
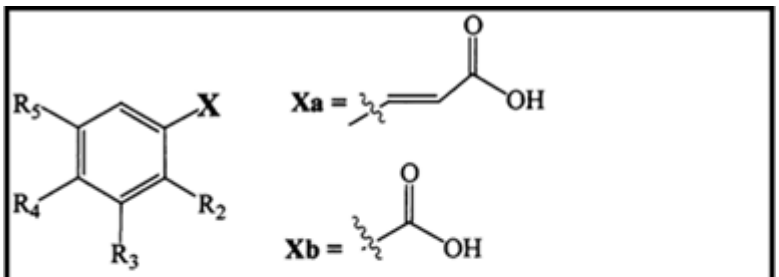


Figure 6. Structures of flavonol aglycones (adapted from: Jaakola and Hohtola, 2010).

The phenolic acids are phenolic compounds that possess one carboxylic acid functionality (**Figure 7**). This group of plant secondary metabolites contain two basic carbon structures: hydroxycinnamic (Xa) and hydroxybenzoic (Xb). Phenolic acids in plants are involved in various functions, including photosynthesis, nutrient uptake, protein synthesis, enzyme activity and allelopathy (Robbins, 2003).

Significant variation in content of hydroxycinnamic acid conjugates between black currant cultivars was reported by Zheng et al. (2012).



R_2	R_3	R_4	R_5	X	
H	H	H	H	a	cinnamic acid
−OH	H	H	H	a	*o*−coumaric acid
H	H	−OH	H	a	*p*−coumaric acid
H	−OH	H	H	a	*m*−coumaric acid
H	$-OCH_3$	−OH	H	a	ferulic acid
H	$-OCH_3$	−OH	$-OCH_3$	a	sinapic acid
H	−OH	−OH	H	a	caffeic acid
H	H	H	H	b	benzoic acid
−OH	H	H	H	b	salicylic acid
H	H	−OH	H	b	*p*−hydroxybenzoic acid
H	$-OCH_3$	−OH	H	b	vanillic acid
H	$-OCH_3$	−OH	$-OCH_3$	b	syringic acid
H	−OH	−OH	H	b	protocatechuic acid
−OH	H	H	−OH	b	gentisic acid
−OH	−OH	−OH	−OH	b	gallic acid
H	$-OCH_3$	$-OCH_3$	H	b	veratric acid

Figure 7. Structures of phenolic acids (adapted from Robbins, 2003).

Black currants also contain proanthocyanins, which are oligomers and polymers of flavan-3-ol units. Proanthocyanins accumulate in different plant tissues and their role, among others, is protection against pests (Oliveira et al., 2014; Jaakola and Hohtola, 2010). They are, together with phenolic acids, flavonoids, sugars and organic acids as well as volatile aroma compounds, responsible for the sensory characteristics of black currants (Sandell et al., 2009).

1.4 Health benefits

Black currants can be consumed fresh, but are also used for processing juice, jams, jelly, syrup, wines and liqueurs (Hummer and Barney, 2002). Black currants are often called “super fruits” because of their high content of health related phytochemicals (Lyall et al., 2009). Several studies

have been conducted to investigate the health promoting properties of black currant berries and of products based on them (Gopalan et al., 2012).

Ascorbic acid (vitamin C) is an essential compound for maintaining health. As a strong antioxidant, vitamin C can mitigate the harmful effects of oxidative stress on cells and tissues (Padayatty et al., 2003). Ascorbic acid is a strong antioxidant and is able to cope with reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide anions (O_2^-) and hydroxyl radicals •OH. This vitamin play a crucial role as an antiscorbutic factor and is involved in biosynthesis of collagen, some peptide hormones, cholesterol, and L-carnitine (Traber and Stevens, 2011; Grosso et al., 2013). Ascorbic acid may also mitigate the results of some cardiovascular problems (Gokce et al., 1999; Riccioni et al., 2012; Juraschek et al., 2012). Moreover, vitamin C may have an important role in prevention of cancer (Lutsenko et al., 2002). Recent studies showed that high doses of ascorbic acid selectively kill KRAS and BRAF mutant colorectal cancer cells (Yun et al., 2015). The difference in structure of ascorbic acid (AA) and dehydroascorbic acid (DHAA) result in contrasting ways of intestinal absorption in mammals. AA is absorbed by the sodium-dependent vitamin C transporter 2 (SVCT2), and DHAA by the facilitated-diffusion glucose transporters, GLUT 2 and 8 (Corpe et al., 2013). After oral application of DHAA (DHAA is converted to AA after absorption), ascorbic acid reach its peak plasma levels faster when compared to AA application (Tsujimura et al., 2008).

Anthocyanins from black currants have positive effects on the cardiovascular system by activation of endothelial nitric oxide synthase (eNOS) (Edirisinghe et al., 2011). Proanthocyanidins from black currant has potential ability to relieve pulmonary inflammation (Hurst et al., 2010). Moreover, positive effects of black currant consumption on the ocular system have been observed in clinical trials (Nakaishi et al., 2000; Matsumoto et al., 2003). Possible anti-cancer properties of black currant berries were rewieved by Folmer et al. (2014), showing the importance of inclusion of this fruits in a balanced diet. In addition, black currant anthocyanins were able to prevent obesity by improving glucose metabolism in model mice (Esposito et al., 2015). Consumption of black currant cold-pressed juice has been reported to improve mood, memory and affect monoaminooxidase enzyme activity, possibly due to properties of the contained phenolic compounds (Watson et al., 2015). Antiviral and antibacterial properties of black currant extracts from leaves and berries have also been reported (Ikuta et al., 2012; Ikuta et al., 2013; Ehrhardt et al., 2013; Haasbach et al., 2014).

1.5 Environmental effects on berry quality

Both genotype and cultivation environment are known to affect chemical composition of black currant, especially ascorbic acid and various phenolic compounds. Environmental conditions, like temperature, solar radiation and precipitation can have significant impact on fruit properties (Lee and Kader, 2000; Vagiri et al., 2014).

Contradictory observations have been reported concerning the impact of field climatic conditions on ascorbic acid accumulation in various fruit crops (Lee and Kader, 2000; Richardson et al., 2004; Walker et al., 2010). A study conducted in Scotland showed positive correlation between summer temperature and ascorbic acid concentration in black currant (Walker et al., 2010). On the other hand, negative correlations between temperature and ascorbic acid accumulation in black currant were reported in a trial conducted in Estonia (Kaldmäe et al., 2013). In addition, observation of lower ascorbic acid accumulation in red and white currants grown in southern Finland, when compared to colder, northern locations, emphasize the importance of specific adaptation ability of species or cultivars to different growth conditions (Zheng et al., 2009a). Black currants grown in simulated climates in growth chambers had a higher vitamin C content in treatment representing more southern (warmer) conditions than in more northern (colder) (Redalen, 1993). It should be kept in mind, however, that accumulation of ascorbic acid in black currant is very sensitive to environmental conditions, showing sometimes almost a two-fold variation between growing seasons (Nes et al., 2012).

Lower summer temperature during fruit ripening have been found to increase accumulation of glucose, fructose and sucrose in black currant berries and other *Ribes* spp. in Finland (Zheng et al., 2009a; b). A similar pattern was observed also for other berries (Wang and Camp, 2000; Richardson et al., 2004). Wang et al. (1993) suggested that sucrose synthase activity decreased with exposure to heat stress.

Influence of temperature on fruit acidity was shown by an experiment conducted in Estonia, where the concentration of organic acids in black currant berries increased in years with higher average temperatures in July (Kaldmäe et al., 2013). On the other hand, a negative correlation between elevated temperature and concentration of malic acid was previously reported for various fruit species (Lobit et al., 2006; Sweetman et al., 2009). Synthesis of malic acid involves an exothermic reaction, and may, therefore be favoured by lower temperatures in comparison to the degradation of the acid (Lobit et al., 2006).

Biosynthesis and accumulation of phenolic compounds is regulated by both genetic and environmental factors (Koes et al., 2005; Jaakola and Hohtola, 2010). Flavonoids are synthesized by the phenylpropanoid pathway thereafter conjugated to sugars such as glucose, rutinose and rhamnose and accumulate in the plant vacuoles as glycosides.

Black currant grown at higher latitude in Finland had lower contents of total flavonols, total anthocyanins, and total phenolic compounds than those grown at lower latitude (Zheng et al., 2012). An experiment conducted in two latitudinally distinct locations in Sweden, revealed that black currant accumulated more total anthocyanins and flavonols when grown in warmer (southern) conditions. However, contradictory patterns in accumulation of individual compounds from both subclasses were observed (Vagiri et al., 2013). Total content of phenolic compounds in red, white and green currants was, however, higher in the north than in the south (Yang et al., 2013).

2. Aims and scope of the present study

The aim of the present study was to provide new knowledge for a deeper understanding of the effects of post flowering environmental conditions on fruit quality and chemical composition of black currant. The relevance of the study was also accentuated by the predicted and ongoing global climatic change (Hartmann et al., 2013; NOAA, 2016).

Previous research on black currant have mainly focused on different weather conditions occurring in latitudinally distinct regions (Zheng et al., 2009b; Vagiri et al., 2013; Yang et al., 2013). Nevertheless, research has also been conducted based on the changes in fruit quality in relation to historical climatic data (Walker et al., 2010; Krüger et al., 2011; Kaldmäe et al., 2013). However, investigations of the relations between climate and different species/cultivars often show only a part of the complete picture, with situations corresponding merely to the local conditions.

To determine the complex relationship between berry chemical content and environmental conditions, two distinct experimental approaches were employed:

1) Correlation analyses of the relationship between berry chemical content and weather conditions in the field (Paper I), and 2) studies on the effects of post-flowering temperature and photoperiod on berry chemical content in plants grown in daylight phytotron compartments with controlled climatic conditions (Papers II, III and IV).

In the first approach, eight years of climate and quality parameters data from a field trial with four black currant cultivars grown at Ås, Norway (59°40'N), were analysed. In the second approach, single-stemmed potted plants of four black currant cultivars where grown post flowering at constant temperatures (12, 18 and 24 °C) and photoperiod (short day, long day and natural light conditions). In addition, as an untreated control, plants of all cultivars were also grown outdoors under ambient light and temperature conditions.

Relations between field climatic conditions (temperature, solar radiation and precipitation) and fruit yield and quality parameters (berry weight, dry matter, soluble solids, titratable acidity, pH, antioxidant capacity, total monomeric anthocyanins, total phenolic compounds and ascorbic acid) were analysed and presented in Paper I.

The impact of post flowering controlled climate (temperature and photoperiod) on selected quality parameters, such as dry matter, soluble solids, titratable acidity, the ratio between soluble solids and titratable acidity, pH and optical density, as well as ascorbic acid, antioxidant capacity, total monomeric anthocyanins and total phenolic compounds were investigated and are presented in Paper II.

Effects of controlled post flowering conditions on ascorbate pool (ascorbic acid and dehydroascorbic acid), individual organic acids and individual sugars were investigated and reported in Paper III, and changes in concentration of individual phenolic compounds under distinct experimental treatments were investigated and described in Paper IV.

3. Materials and methods

3.1 Plant material and experimental design

3.1.1 Field experiment

The first experiment (Paper I) was conducted during the years 2005 to 2012 in an experimental field at the Norwegian University of Life Sciences at Ås, Norway (59°39′N-10°45′E). Small plants of the Scottish cultivars 'Ben Hope' ('Westra' x (238/36 x EM21/15)) and 'Ben Tron' (ND12/26 x (('Vistavotnjaja' x ('Mendip Cross' x '*R. dikuscha'*) x ('Goliath' x 'Øjebyn')) x 'Westra'), and the Norwegian cultivars 'Kristin' ('Ben Tron' x L I 11/46-85 ('Hedda' x EM 1428/70)) and 'Varde Viking' ('Narve Viking' x 'Titania') were planted in 2001. The experiment had a randomized

block design with three replicates, each containing one bush of each cultivar. No irrigation or plant protection sprayings were applied, and fertilization and pruning was performed according to standard recommendations. Berries were hand harvested (July 27-August 5, depending on year) when regarded as fully ripe, immediately frozen and stored at -20 °C until processed and analysed.

Climatic variables were described by the sums of individual meteorological parameters in the particular year. For temperature, sums of average daily temperatures for the periods 5, 10 and 15 days before harvest, as well as the sums of daily temperatures in May, June and July and the sums of two-month temperatures (May and June and June and July) were calculated. Sums of photosynthetically active radiation (PAR) for 5, 10 and 15 days before harvest, as well as sums of PAR from May, June and July and the sums of June and July were calculated. In addition, calculation of the UV radiation 5, 10 and 15 days before harvest and in July were performed, and sums of precipitation during the months of May, June and July, and in June and July together, were likewise calculated. Historical climatic data was obtained from the local meteorological station (NMBU Report, 2013). Correlation analysis was performed to assess the statistical relationship between climatic data and fruit quality parameters.

3.1.2 Experiment in controlled environment

For the experiment in controlled (phytotron) conditions (Paper II-IV) single-stemmed potted plants of four black currant cultivars were produced as described by Sønsteby and Heide (2011). The use of such single stemmed plants eliminated any biased effects due to plant size, age, and number of branches. In addition, the use of such uniform plant types allowed a good randomization and organisation of the plants on trolleys in the phytotron, giving an experiment with high statistical power. The chosen cultivars originated from breeding programs located at contrasting geographical latitudes, and included the high-boreal Russian cultivar 'Imandra' ('Primorsky Champion' x 'Pecherskaya'), origination from the Kola Peninsula (67º30'N), 'Hedda' ('Ôjebyn' x 'Melalahti') and 'Narve Viking' ('Ben Tron' x SCRI C2/1/62) from the Norwegian breeding program at Ås (59º40'N) and 'Ben Tron' (ND12/26 x (('Vistavotnjaja' x ('Mendip Cross' x *R. dikuscha*) x ('Goliath' x 'Ôjebyn')) x 'Westra') from the Scottish breeding program in Dundee (56º30'N). Semi-softwood cuttings (5 - 6 cm) from one-year-old shoots were prepared from virus indexed bushes in a field trial in April 2013. The cuttings were stored at 2 °C for 1 week, placed in water at 20 °C for 2 weeks, and then rooted in small pots. A peat based growth medium was

used throughout the experiment. After rooting, the cuttings were potted in 3 L pots and kept in a greenhouse at 20 °C and 24 h light, until they had produced ≥ 12 leaves. The plants were then moved outdoors at Ås, Norway (59º40′N, 10º45′E), in late June, 2013, and kept under natural conditions until growth cessation in late November. After leaf abscission and hardening outdoors in November, the plants were moved into a cool storage at 0 °C for controlled over-wintering.

In spring 2014, plants were moved outdoors and placed in four East-West oriented rows (spacing 0.3 m within the row, and 1.5 m between rows), in a randomized block design. After flowering and pollination, approximately three weeks before full ripeness, the plants were moved into the phytotron (59º40′N, 10º45′E) (**Figure 8**), and exposed to combinations of three temperatures (12, 18, 24 ºC) and three photoperiod conditions: 10 h (10 h light 08:00 h to 18:00 h and 14 h dark = short day), 10 + 3 h (10 h light + 3 h night interruption in the middle of the 14 h dark period = long day) and natural photoperiod (full daylight = 18-19 h long day). The daylight compartments were maintained at constant temperatures (± 1 ºC) and a water vapour pressure deficit of 530 Pa was maintained at all temperatures. All plants received 10 h of summer daylight from 08:00 h to 18:00 h in the daylight compartments. During the night interruption treatment, low-intensity light (approx. 7 µmol quanta m^{-2} s^{-1}) from incandescent lamps (70 W) was used, adding less than 0.5 % to the total daily light integral. The full daylight treatment provided about 9 % higher total daily light integral, when compared to the short day and short day with night interruption treatments. Each treatment combination had four replications, with two plants of each cultivar on a separate trolley. The trolleys were randomly distributed in the phytotron compartments by the every-day movements to and from the photoperiod treatment rooms. In addition, groups of eight plants of each cultivar remained under field conditions as a control. In all treatments, berries were harvested when fully ripe, based on visual assessment of colour and berry softness (**Figure 9** and Paper II).

Figure 8. Single stem plants of black currants organised on trolleys in daylight compartments in the phytotron. Each trolley contained two plants of each cultivar representing one of four treatment replications. (photo: T. L. Woznicki)

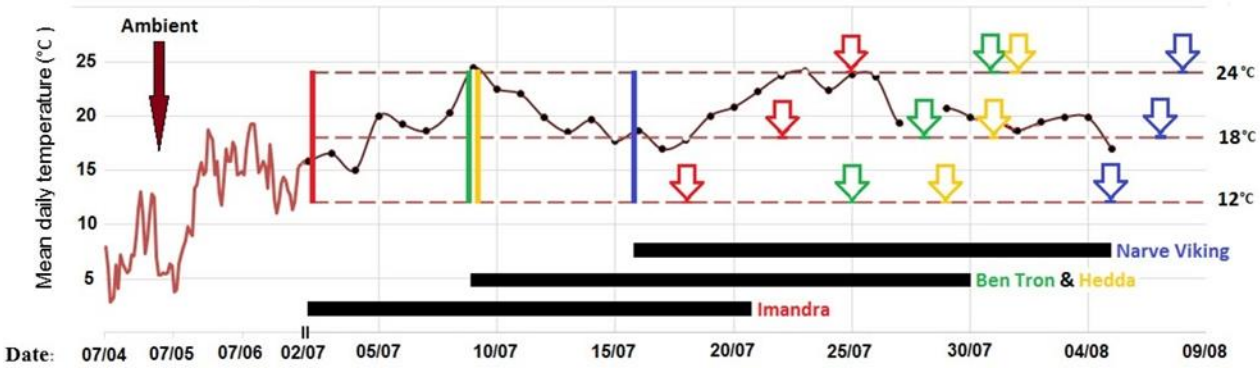


Figure 9. Collation of ambient and controlled temperature during the experiment, together with the treatment durations for each cultivar in each temperature. The vertical coloured lines represent the commencement of treatment for the individual cultivars, and arrows indicate harvest dates. The black lines below represent treatment durations for the ambient control. The wavy line with black dots denote time course of the ambient temperature. Blue – 'Narve Viking', green – 'Ben Tron', yellow – 'Hedda', red – 'Imandra'.

3.2 Chemical analyses

3.2.1 Soluble solids, pH, titratable acidity, dry matter content, antioxidant capacity, total monomeric anthocyanins, and total phenolic compounds

Determinations of soluble solids (SS), pH, titratable acidity (TA) and dry matter content (DM) are described in detail in Paper I and II. For analyses of antioxidant capacity (AOC, determined as Ferric Reducing Antioxidant Power, the FRAP assay), total monomeric anthocyanins (TMA), and total phenolic compounds (TP) a KoneLab 30i (Thermo Electron Corp., Vantaa, Finland) analyser was used. Analysis of AOC was performed according to Benzie and Strain (1996), TMA analysis was performed by the pH differential method (Giusti and Wrolstad, 2005) and TP was determined using the Folin−Ciocalteu method (Singleton et al., 1999) (Paper I and II).

3.2.2 Ascorbic acids

Samples for ascorbic acid analyses were prepared as described by Wold et al. (2004), and ascorbic acid determination in Paper I was performed according to the method described by Williams (1976). Vitamin C concentrations presented in Paper II and III were determined as L-ascorbic acid (AA) and dehydroascorbic acid (DHAA) according to Aaby et al. (2007). Concentration of DHAA was determined by analysing AA in a separate sample after its reduction. The amount of DHAA was the difference between the samples.

3.2.3 Sugars and organic acids

The concentration of sugars and organic acids was determined using an HPLC analyser equipped with a DAD and a refractometer index (RI) detector as described in Paper II.

3.2.4 Individual phenolic compounds

Sample preparation and analysis of individual phenolic compounds (anthocyanins, flavonols and hydroxycinnamic acids) were conducted using HPLC-DAD-MS^n spectrometry according to Remberg et al. (2010) (see Paper IV).

3.3 Statistical analysis

All statistical analyses were performed using a Minitab 16 statistical software package. Pearson correlation analysis was performed to investigate the relationship between weather conditions and fruit chemical composition (Paper I) as well as the relationship between individual sugars and concentration of ascorbic acid (Paper III). Principal component analysis (PCA) was used to find relations between climatic variables and fruit chemical composition (Paper I), as well as to show the differences in concentrations of individual phenolic compounds between the investigated cultivars (Paper IV). To analyse the data from the experiment conducted under controlled environment (Paper II, III and IV) a three-factor fixed effect General Linear Model (GLM), together with Tukey's multiple comparison test with significance levels $\alpha = 0.05$ was used.

4. Results

4.1 Relationship between berry chemical content and weather conditions in the field (Paper I)

Climatic conditions during the growing season had significant impact on all investigated black currant quality parameters except titratable acidity. Yield was positively correlated with precipitation during fruit development, whereas a negative correlation was observed between yield and summer temperatures and radiation, as well as between berry weight and summer temperature. Black currant berries had higher concentration of soluble solids and higher dry matter content in years with low precipitation, high summer temperatures and photosynthetically active radiation (PAR). The berries had higher pH in years with higher summer temperature and low precipitation. Antioxidant capacity (AOC) was mainly positively correlated with photosynthetically active radiation during the ripening, while correlations between temperature and AOC were not consistent. A negative correlation between temperature and total monomeric anthocyanins, as well as a positive correlation between photosynthetic active radiation and total monomeric anthocyanins were also observed. The results of the field experiment revealed a strong impact of environmental conditions on fruit total phenolic content (negative correlation with temperature and PAR and positive with precipitation). Ascorbic acid concentration in berries was strongly influenced by environmental conditions during ripening, with mainly negative correlations with summer temperature and PAR and positive correlation with precipitation during the summer. The

simplified general results are summarized in **Figure 10** using Pearson's correlation coefficients between sums of average temperature and precipitation in three months (Mai, June and July) before harvest and the berry parameters: yield, berry weight, dry matter, soluble solids, pH, total monomeric anthocyanins, total phenolics and ascorbic acid.

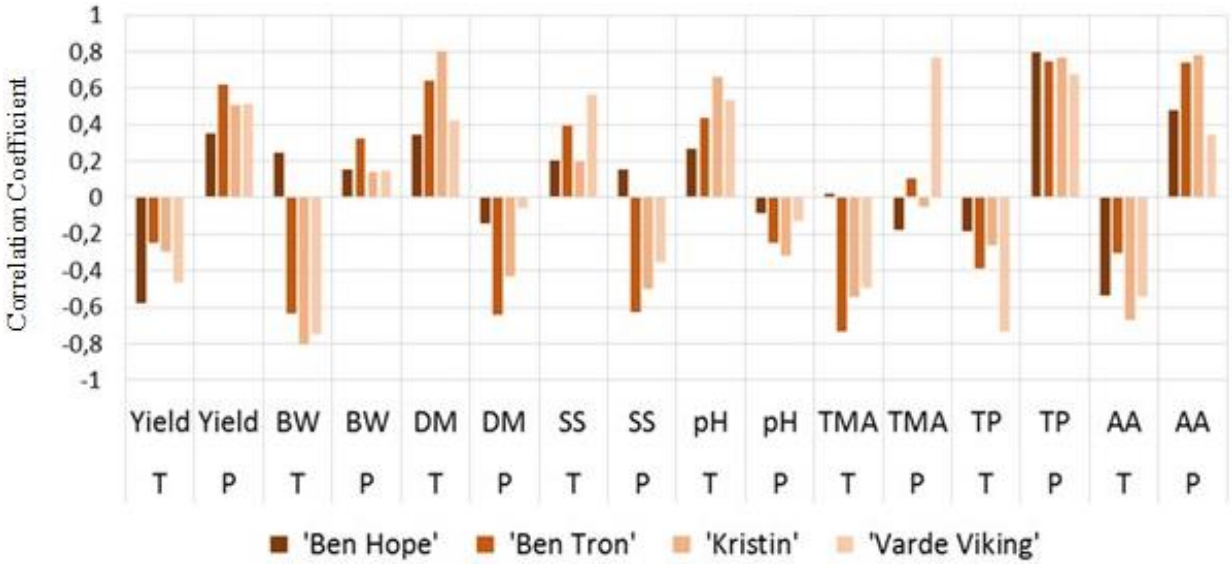


Figure 10. Pearson correlation coefficients between berry parameters: yield, berry weight (BW), dry matter (DM), soluble solids (SS), pH, total monomeric anthocyanins (TMA), total phenolics (TP), ascorbic acid (AA), and the climatic variables T (temperature Mai-July) and P (precipitation Mai-July). (Recalculated data from Paper I)

Significant differences between cultivars were observed for all investigated black currant parameters (**Figure 11**). The most prominent example is ascorbic acid, with a two-fold difference between 'Ben Hope' and 'Kristin'.

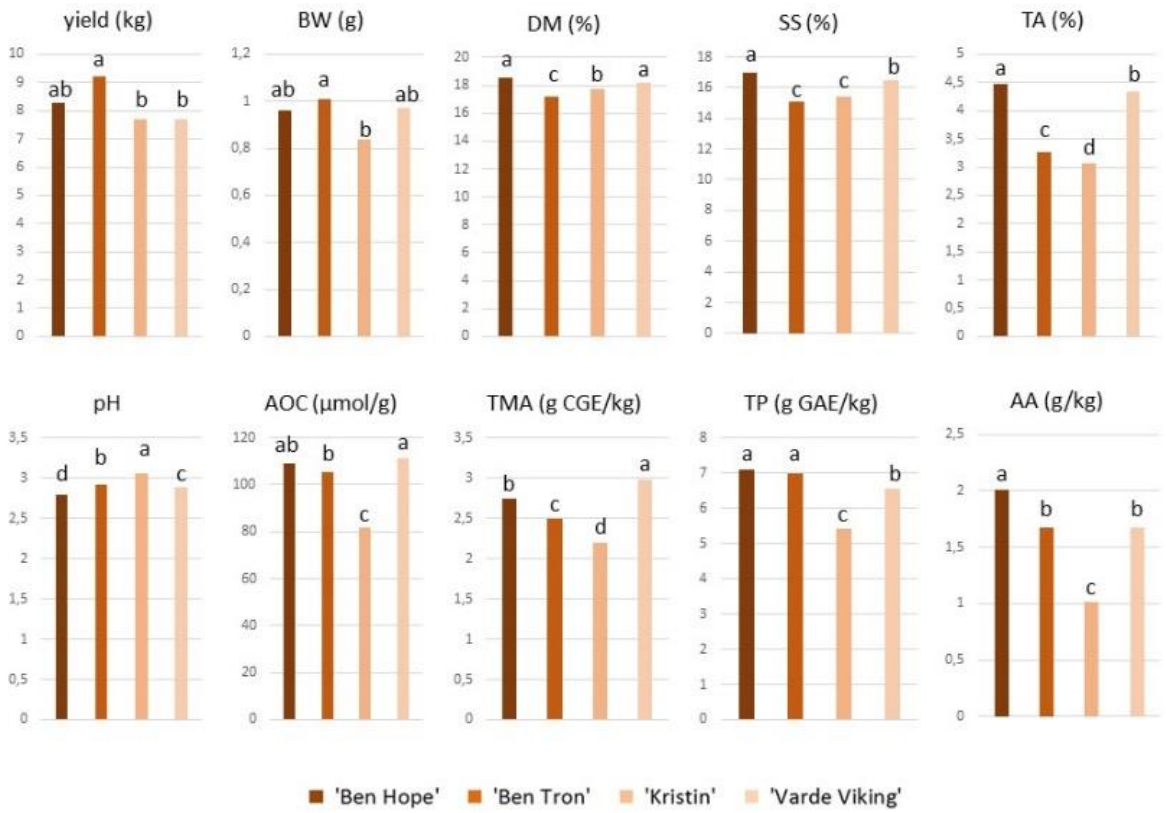


Figure 11. Quality parameters of four black currant cultivars during the eight year field experiment. Data represents eight year means and are given on a fresh weight basis. Means that do not share the same letter are significant different at $p < 0.05$ level. (Paper I)

4.2 Effects of temperature and photoperiod in a controlled (phytotron) environment (Papers II, III and IV)

Soluble solid concentrations in berries were relatively stable across the various temperature and photoperiod conditions in the phytotron, while dry matter percentage increased at the highest temperature (Paper II). Titratable acidity increased with increasing temperature over the 12-24 ºC temperature range, whereas ratio between soluble solids and titratable acidity decreased (Paper II). Under controlled climatic conditions, temperature had little or no effect on pH of the berries (Paper II).

The concentration of total monomeric anthocyanins in the berries was highest at 18 °C, with both higher and lower temperatures resulting in lower accumulation. Total phenolics in berries ripened under controlled climate conditions remained relatively stable across the different temperature and light regimes, while antioxidant capacity was reduced at 24 °C (Paper II).

Accumulation of both forms of ascorbic acid (reduced and oxidized) increased with decreasing ripening temperature (Paper II and III), while the ratio between AA and DHAA increased with elevated temperature (**Figure 12** and Paper III).

The concentration of citric acid increased with increasing ripening temperature (**Figure 12** and Paper III), while malic and shikimic acids showed the opposite temperature trend. The concentration of quinic acid was relatively stable over the 12-24 °C temperature range, with the highest accumulation at 18 °C (**Figure 12** and Paper III).

The individual sugars (glucose, fructose and sucrose) increased significantly with decreasing temperature, giving a reduction of 27 % in total sugars when comparing 24 and 12 °C. The temperature effect was larger on the hexoses than on sucrose (**Figure 12** and Paper III).

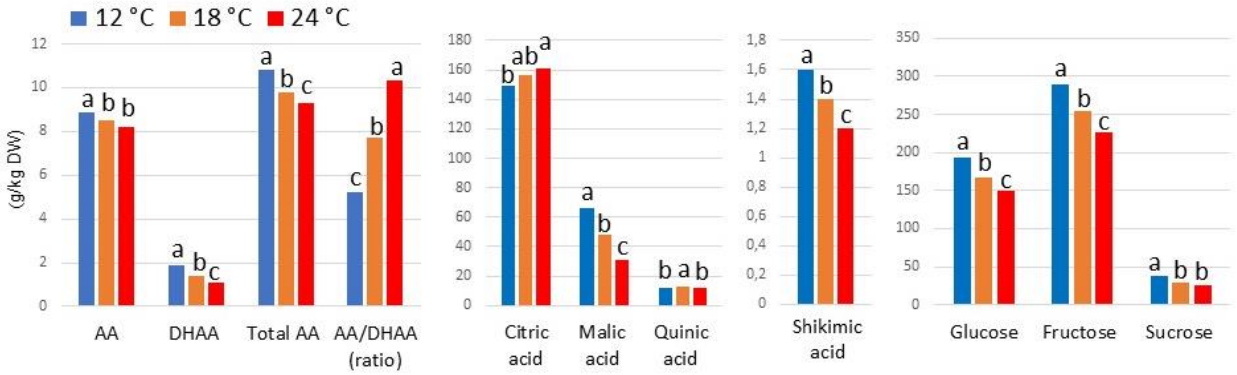


Figure 12. Concentration (g/kg DW) of ascorbic acids (AA, DHAA), organic acids and sugars under controlled temperature conditions. Data are means of four cultivars and all daylength conditions. Means that do not share the same letter are significant different at $p < 0.05$ level. (Paper III)

Influence of controlled climatic conditions on individual phenolic compounds in the black currant berries are presented in Paper IV. Analysis of individual anthocyanins, flavonols and hydroxycinnamic acids revealed different, sometimes opposite patterns of accumulation for compounds in the same subclass across the temperature regimes (Paper IV). Increased temperature over the 12-24 °C range caused a significant increase in the concentrations of delphinidin-3-glucoside, delphinidin-3-(6”-coumaroyl)-glucoside and cyanidin-3-(6”-coumaroyl)-glucoside, while the opposite trend was observed for cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside (**Figure 13**). The highest accumulation of delphinidin-3-rutinoside was observed at 18 °C.

Natural summer daylength conditions promoted accumulation of several anthocyanins such as delphinidin-3-glucoside, delphinidin-3-rutinoside, delphinidin-3-6-coumarylglucoside and cyanidin-3-6-coumarylglucoside as well as of total anthocyanins (Paper IV).

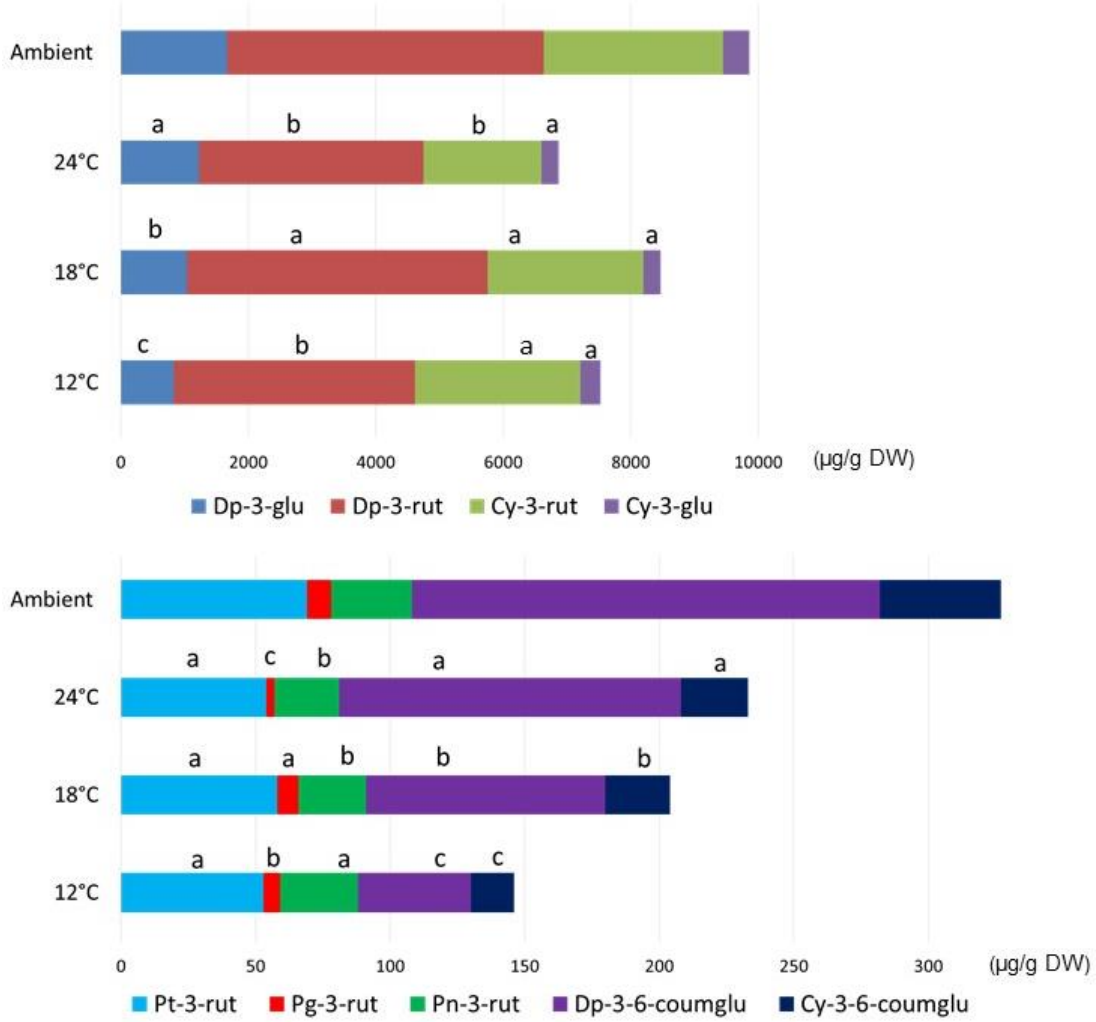


Figure 13. Concentration (µg/g DW) of individual anthocyanins under controlled and ambient temperature conditions. Data are means of four cultivars and all daylength conditions. Means that do not share the same letter are significant different at $p < 0.05$. Abbreviation used: Dp = delphinidin, Cy = cyanidin, Pt = petunidin, Pg = pelargonidin, Pn = peonidin, glu = glucoside, rut = rutinoside, coumglu = coumaroylglucoside. (Paper IV)

High ripening temperature promoted accumulation of myricetin-3-glucoside, while myricetin-3-malonylglucoside and quercitin-3-malonylglucoside showed the opposite trend. For myricetin-3-rutinoside and total flavonols, the highest concentrations were found at 18 °C. (**Figure 14**, Paper IV). Higher accumulation of myricetin-3-rutinoside and 3-glucoside as well as quercetin-3-glucoside under the treatment with higher daily light integral was also observed (Paper IV).

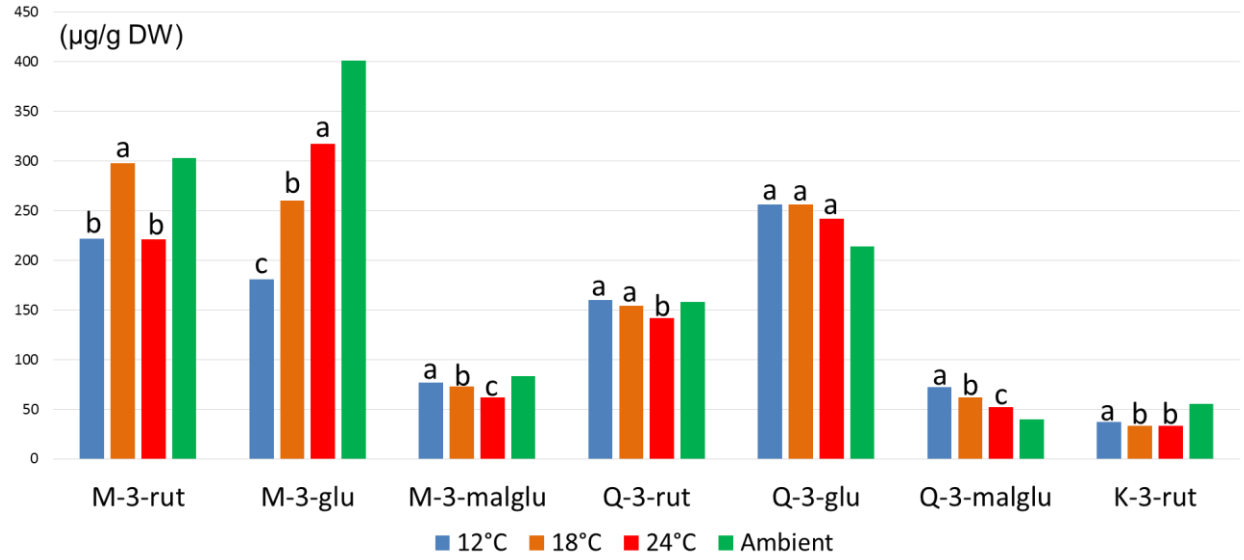


Figure 14. Concentration (µg/g DW) of individual flavonols under controlled and ambient temperature conditions. Data are means of four cultivars and all daylength conditions. Means that do not share the same letter are significant different at $p < 0.05$ level. Abbreviation used: M = myricetin, Q = quercetin, K = kaempferol, rut = rutinoside, glu = glucoside, malglu = malonylglucoside. (Paper IV)

Accumulation of the majority of the hydroxycinnamic acid derivatives was promoted by low ripening temperature across the entire temperature range. The only exception was an identified derivative of caffeic acid. The concentrations of hydroxycinnamic acids found in the berries were generally not significantly affected by different daylength conditions (Paper IV).

The concentrations of all anthocyanins and two flavonols (myricetin-3-glucoside and kaempferol-3-rutinoside) were lower in controlled climate compared to the ambient control (**Figure 13** and **14**). However, no such response was noted for the hydroxycinnamic acids (Paper IV).

In general, there were no significant differences between berries ripened under different photoperiodic conditions with identical daily light energy (Paper II-IV).

The experiment conducted in controlled climate revealed several intriguing patterns for accumulation of various quality related substances in berries of different cultivars. Based on results described in Paper II and III, a principal component analysis was applied to visualize the general differences in berry composition among the investigated cultivars (**Figure 15**). The score plot showed a clear separation of the four cultivars studied, while the loading plot revealed positive correlation between compounds that are close to each other, and negative correlation between

compounds that are symmetrically distant on the loading plot area. In addition, when score plot and loading plots are superimposed, information on the cultivar chemical composition can be obtained. When a berry chemical compound (loading plot) is plotted close to a cultivar (score plot), this indicates that this compound had relatively high concentration in this particular cultivar.

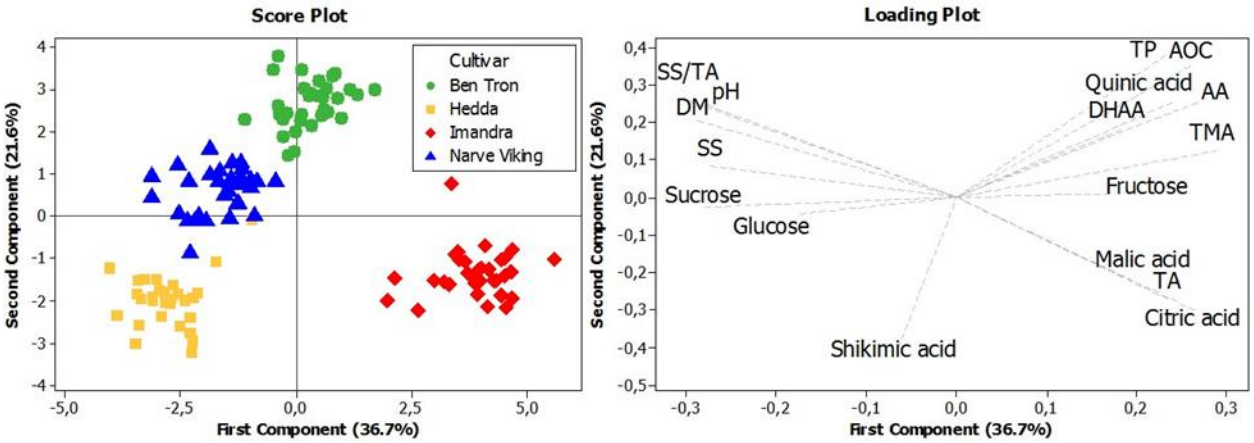


Figure 15. Score plot and loading plot of principal component analysis (PCA) based on the correlation matrix of results presented in Paper II and III. The first two principal components represented 36.7 and 21.6 % of the variance, respectively.

Berry size varied among the investigated cultivars, with 'Imandra' having the lowest average berry weight (1.4 g), followed by 'Narve Viking' (1.5 g), 'Ben Tron' (1.5 g) and 'Hedda' (1.9 g) (Paper II). Berries of 'Ben Tron' had the highest antioxidant capacity, concentration of total phenolics, and the lowest titratable acidity. 'Hedda' was characterized by having the lowest antioxidant capacity, total monomeric anthocyanins and total phenolics. 'Imandra' berries had the highest titratable acidity and total monomeric anthocyanins, and the lowest dry matter content, soluble solids and soluble solids/titratable acidity ratio. On the other hand, berries of 'Narve Viking' had the highest soluble solids and dry matter content.

A remarkable stability of dry matter content across the 12-24 °C temperature range was observed in berries of 'Hedda' and 'Imandra'. In addition, a stable accumulation of soluble solids and total phenolic compounds was found in 'Narve Viking' (Paper II).

'Hedda' had the lowest concentration of both reduced and oxidised forms of ascorbic acid (Paper III). This cultivar had however, the highest concentration of glucose as well as total sugars.

On the other hand, ‘Imandra’ had the lowest concentration of sucrose and the highest concentration of fructose. In addition, berries of this cultivar had the highest concentration of most organic acids (citric, malic and quinic) and the two forms of ascorbic acids. The highest sucrose concentration was observed in berries of ‘Narve Viking’ (Paper III). The AA/DHAA ratio varied from 5.6 in cultivar ‘Hedda’ to 10.3 in ‘Narve Viking’.

Interestingly, ‘Imandra’ was characterised by a remarkable stability in accumulation of ascorbic acid and dehydroascorbic acid across the 12-24 °C temperature range. In addition, high stability in accumulation of quinic acid was noted for ‘Ben Tron’. Decomposition of data also revealed a relatively stable concentration of sucrose in berries of the cultivars ‘Ben Tron’ and ‘Narve Viking’ across the temperature regimes (Paper III).

The principal component analysis presented in Paper IV indicated several differences in composition of individual phenolic compounds among the investigated cultivars. Berries of ‘Ben Tron’ were characterized mainly by high concentrations of anthocyanins, and high total flavonol content. Berries of ‘Hedda’ were characterized by the highest concentration of rutin (quercetin-3-rutinoside), while ‘Imandra’ accumulated high amounts of cyanidin-3-(6’’-coumaroyl)-glucoside, as well as myricetin-3-glucoside and myricetin-3-malonylglucoside. ‘Narve Viking’ accumulated the highest amounts of kampferol-3-rutinoside.

‘Ben Tron’ showed high stability in accumulation of petunidin-3-rutinoside as well as total anthocyanins across the 12-24 °C temperature range. The accumulation of two flavonols (quercetin rutinoside and quercetin glucoside) in the berries of this cultivar was not affected by post flowering temperatures. Berries of ‘Hedda’ had high stability in accumulation and therefore similar concentrations of cyanidin-3-glucoside, petunidin-3-rutinoside as well as total anthocyanins across the temperature range. The accumulation of caffeoyl quinic acid in this cultivar was not affected by ripening temperature. A stable accumulation of peonidin-3-rutinoside and caffeoyl quinic acid was observed in ‘Imandra’ while ‘Narve Viking’ also accumulated cyanidin-3-glucoside, peonidin-3-rutinoside and quercetin-3-rutinoside independently of the temperature regime (Paper IV).

5. Discussion

The effect of climate conditions on berry yield and quality under field conditions are sometimes difficult to evaluate due to co-variation between several interacting factors in natural environment. For example, significant correlation between photosynthetically active radiation and some of the quality parameters may also reflect an influence of temperature or drought as factors associated with high solar radiation. Similar situations can be described for precipitation which is often associated with cooler days and lower photosynthetically active radiation in period of full cloudiness (reversed correlation patterns for temperature and precipitation in **Figure 10**).

Nevertheless, analysis of correlation over a relatively long period (eight years) gave us an opportunity to observe the most characteristic relations between black currant quality and weather conditions in a Nordic climate. Earlier studies have reported positive correlations between temperatures in July and soluble solids in black currant (Zheng et al., 2009b; Kaldmäe et al., 2013), and are in general agreement with our results from the field experiment (Paper I).

Results from Finland confirm our general observations for total phenolic compounds under field conditions, indicating a negative impact of high radiation and high temperature during the Nordic summer on accumulation of major phenolic compounds in the berries (Yang et al., 2013). In addition, decreased antioxidant capacity under elevated temperature have been previously observed for strawberries (Kalt et al., 2001) and black currants (Remberg et al., 2012), however, our results were not consistent for this parameter (Paper I).

A positive impact of low post flowering temperature on accumulation of total monomeric anthocyanins, as well as a positive correlation between photosynthetic active radiation and total monomeric anthocyanins, was observed in both of our experiments (Paper I and II). Flavonoid-related biosynthesis genes are upregulated by light (Azuma et al., 2012) and this may, at least in part, be the explanation of the observed results. In grapes, accumulation of total anthocyanins was promoted by moderate temperature (20 °C compared with 30 °C), while at 35 °C, inhibition of mRNA transcription and anthocyanin degradation was observed (Yamane et al., 2006; Mori et al., 2005). It should be kept in mind, however, that black currant, being an understory shrub originating from Northern and Central Europe, may react differently from grapes under environmental stress. Nevertheless, molecular genetic analysis of apples (originating from temperate regions) also revealed that low temperature during ripening is an inducing factor for the expression of key genes controlling anthocyanin biosynthesis, such as CHS (chalcone synthase), ANS (anthocyanidin

synthase), and UFGluT (UDP-glucose: flavonoid 3-O-glucosyl-transferase) (Ubi et al., 2006). Therefore, increased accumulation of anthocyanins seems to be a general response of fruits ripened at low temperatures, while decreasing at heat stress temperatures.

It should also be kept in mind, that analysis of total monomeric anthocyanins often shows only a part of the complete picture. Results presented in Paper IV, show that accumulation of different anthocyanins may have opposite patterns across the temperature range, or have specific temperature optima. This emphasizes the importance of careful examination of such analytical results.

Ascorbic acid concentration in black currants, both in the field and under controlled climate conditions, was similarly reduced by elevated temperature during ripening (Paper I and III). Reduced accumulation of ascorbic acid under higher ripening temperature was also observed during an experiment with the black currant cultivars 'Ben Sarek' and 'Ôjebyn' (Redalen, 1993). Similarly, negative correlation between temperature and ascorbic acid accumulation was reported from a trial conducted in Estonia (Kaldmäe et al., 2013). However, in contrast to these results, temperature and total solar radiation from April to July was positively correlated with ascorbic acid concentration in black currants grown in Scotland (Walker et al., 2010). In addition, the authors observed significant variation in accumulation of ascorbic acid in black currants grown in distinct locations in the UK during the same growing season. This emphasize high sensitivity of ascorbic acid accumulation due to local climate, soil properties and genetic background of the plants.

Ascorbic acid (AA) in black currant is produced *in situ* in the berries via the L-galactose pathway, and its accumulation is regulated by post translational and post transcriptional mechanisms (Hancock et al., 2007; Walker et al., 2010). Ascorbic acid accumulation is dependent on its biosynthesis, oxidation and recycling (Ioannidi et al., 2009). It is known, that ascorbate peroxidase (APX) catalyses the oxidation of AA into monodehydroascorbate (MDHA), a radical which is rapidly converted into AA and dehydroascorbate (DHAA). Monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) are used for the enzymatic regeneration of AA from MDHA and DHAA (Jimenez et al., 1997). The ratio between reduced and oxidized forms of ascorbic acid depends on the redox state of the plant cells (Gallie, 2013) and can be influenced by environmental conditions, which lead to changes in

the expression levels of the ascorbic acid biosynthetic genes, as well as the ascorbate recycling genes (MDHAR and DHAR) (Liu et al., 2015).

The experiment conducted in controlled climate showed a positive correlation between citric acid and titratable acidity ($r = 0.67$, $p < 0.001$). This is as expected since citric acid was the predominating acid in the black currant cultivars. In accordance with our findings (Paper III), a study conducted in Estonia (Kaldmäe et al., 2013), showed that elevated temperature increased citric acid accumulation in black currant. Moreover, Remberg et al. (2010) also described similar pattern for 'Glen Ample' raspberries grown under controlled environment conditions. In accordance with our findings, a negative impact of elevated temperature on accumulation of sugars in black currants and other *Ribes* spp. was also observed by Zheng et al. (2009a, b).

The reason for a relatively weak temperature effect on soluble solids (which includes the carbohydrates, organic acids , proteins, fats and minerals) under controlled climate condition (Paper II) might be the simultaneous change in the balance between components of soluble solids under the 12-24 ºC temperature range. The observed decrease in accumulation of sugars under elevated temperature together with increasing accumulation of citric acid (Paper III) supports this assumption and may partly explain the stable soluble solids content (Paper II). The contrasting and highly significant impact of ripening temperature on accumulation of both sugars and citric acid, indicate that black currants grown under warmer conditions may, in general, be less attractive for fresh consumption.

The patterns of accumulation of individual phenolic compounds in berries ripened in controlled climate (Paper IV) showed some analogies with the results of previously conducted field experiments. An increased accumulation of delphinidin-3-glucoside with increasing temperature is in agreement with previous findings with black currants grown in Finland, where the concentration of this anthocyanin showed a positive correlation with summer temperature (Zheng et al., 2012). The observed accumulation of delphinidin-3-glucoside and cyanidin-3-rutinoside in our experiment, are in agreement with a Swedish study on black currants grown at two latitudinal locations with cool temperatures (northern part of Sweden) vs. a warmer location (southern part of Sweden) (Vagiri et al., 2012), and thereby confirm the role of temperature as a factor influencing the accumulation of individual phenolic compounds.

The higher accumulation of several individual anthocyanins and flavonols under natural summer daylength (Paper IV) may be explained by the increased expression of flavonol synthase

gene (FLS) (Downey et al., 2004), as well as a light driven general upregulation of flavonoid-related biosynthesis genes (Azuma et al., 2012).

Black currants ripened under ambient conditions was characterized by higher accumulation of all anthocyanins and some of the flavonols when compared to the berries ripened under controlled climatic conditions, regardless of temperature (**Figure 12** and **13**). The reason for this is not fully clear. It seems very likely, however, that reduced UV radiation in the phytotron might have contributed to reduced activity of the key enzymes regulating biosynthesis of flavonoids (to a certain extent both phenylalanine ammonia lyase and chalcone synthase) due to UV-B radiation screening by the glass barrier (Jaakola and Hohtola, 2010). No such response was noted for hydroxycinnamic acid, most evidently due to a strong upregulation of chalcone synthase by UV-B radiation, which act specifically on flavonoid production, not affecting biosynthesis of hydroxycinnamic acids (Jaakola and Hohtola, 2010). Furthermore, the lack of chilling night temperature under the controlled climatic conditions might have been an additional factor causing lower accumulation of flavonoids. Thus, Lin-Wang et al. (2011) observed that only a single night with chilling temperatures upregulated the expression of MYB10 transcription factor in apple skin, and hence enhanced the biosynthesis of anthocyanins.

Comparison of the impact of post flowering temperature on selected fruit quality parameters across the two approaches (field and controlled environment) (Paper I and II) is presented in **Figure 16**. The patterns of correlation between temperature in July (Paper I) and fruit quality parameters are in general agreement with the results obtained under controlled climatic conditions (Paper II). Calculations based on meteorological data (NMBU, 2013) revealed temperature conditions comparable to the controlled climatic conditions. Thus, in the years 2005-2012 the mean temperature in July varied from 15 to 20 °C, while the mean temperature during the last two weeks before harvest varied from 14 to 21 °C. This shows that the range of temperatures selected for the phytotron experiment are quite representative for the natural conditions and may, to some extent, reflect the patterns of changes of chemical composition during the fruit ripening in the northern temperate and subarctic zones. For example, vitamin C increased with decreasing temperature regime (Paper III) and this observation supports the impact of ripening temperature on ascorbic acid accumulation under field conditions described in Paper I **(Figure 10).**

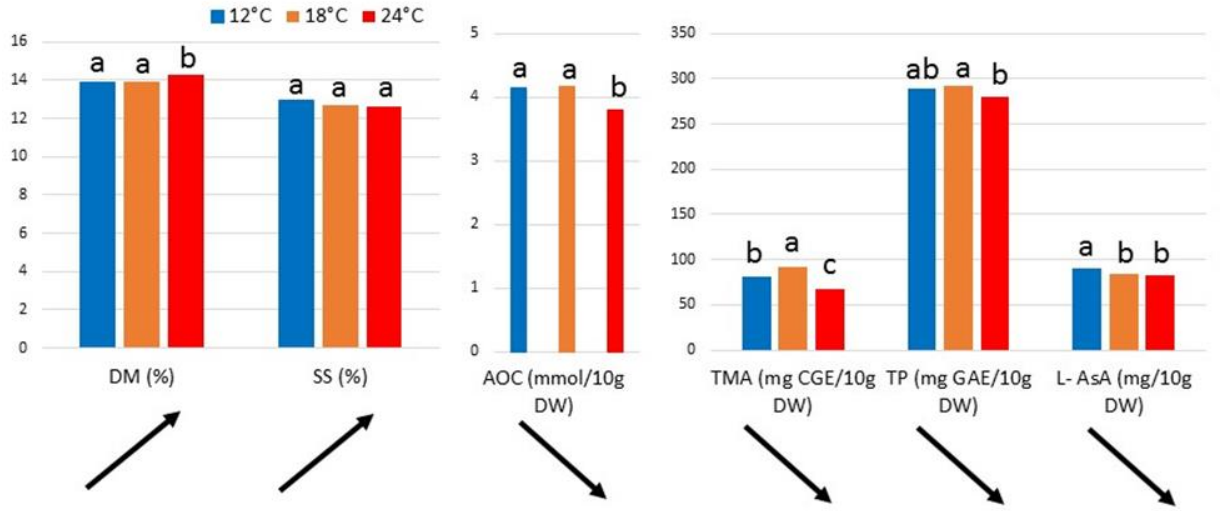


Figure 16. Comparison of effects of constant 12, 18 and 24 °C (Paper II) vs. ambient outdoor temperature in July on selected black currant quality parameters. Arrows represents correlation trends: upward = positive, downward = negative (Paper I). Means that do not share a letter are significant different at $p < 0.05$ level. Abbreviations used: DM = dry matter; SS = soluble solids; TA = titratable acidity; AOC = antioxidant capacity; TMA = total monomeric anthocyanins; TP = total phenolics; L-AsA = ascorbic acid.

Many authors have reported differences in chemical composition between commercial black currant cultivars (Krüger et al., 2011; Zheng et al., 2012; Vagiri et al., 2013). Genotypic differences were also observed in our studies both in the field and in controlled climate. Not all cultivars showed equal accumulation of the investigated compounds. In addition, significant cultivar x temperature interactions demonstrate differences in the cultivars' reaction norms in production or accumulation of secondary plant metabolites across different temperature regimes.

The black currant cultivars used in our studies have also been examined by others. Several studies confirm the good yielding capacity of 'Ben Tron' (Krüger et al., 2011; Nes et al., 2012). A relatively high concentration of ascorbic acid in 'Ben Hope' was also reported by Walker et al. (2010). In addition, the content of ascorbic acid in 'Ben Hope' was relatively high when compared to other cultivars like 'Ben Lemond' or 'Joniniai', known to be rich in ascorbic acid (Pedersen, 2008). Krüger et al. (2011) and Nes et al. (2012) previously reported low concentrations of ascorbic acid in 'Kristin' which is in agreement with our study (Paper I). The previously described low ascorbic acid concentration in 'Hedda' (Heiberg et al., 1992) also concurs with our observations (Paper III).

‘Ben Tron’ had higher antioxidant capacity than ‘Narve Viking’ when grown in the controlled climate (Paper II). However, an experiment conducted in Germany (Krüger et al., 2011), revealed that ‘Narve Viking’ had higher antioxidant capacity than ‘Ben Tron’, apparently due to differences in ripening conditions or discrepancy between analytical methods (TEAC vs. FRAP). ‘Narve Viking’ had the highest soluble solids content among the examined cultivars (Paper II). Nevertheless, when compared to other cultivars suitable for organic production, the soluble solids content in ‘Narve Viking’ from our study was relatively low (Pedersen, 2010).

The present results confirm the importance of genotype as the main factor influencing the concentration of health related compounds in black currant berries (Krüger et al., 2011; Vagiri et al., 2013). Significant variation between different black currant cultivars in the accumulation of individual phenolic compounds is in agreement with previous reports (Rumpunen et al., 2012). However, at least for phenolic compounds, differences in berry size may be partly responsible for the varying concentrations reported for black currant cultivars. The most abundant phenolic compounds in black currants (anthocyanins and flavonols) are found mainly in the berry skin. Larger berries have a lower skin to volume ratio, and therefore, cultivars with large berries may have lower overall concentrations of phenolic compounds (Krüger et al., 2011).

The field experiment revealed many significant cultivar x year interactions, indicating varying responses of different cultivars to particular weather conditions (Paper I). For example, the concentration of total monomeric anthocyanins showed opposite correlation with UV-radiation for the cultivars ‘Ben Hope’ and ‘Varde Viking’. In addition, a positive correlation between total monomeric anthocyanins and photosynthetically active radiation was observed for all investigated cultivars, except for ‘Varde Viking’.

An intriguing example of contrasting cultivar x temperature interactions in the accumulation of one of the flavonols (quercetin glucoside) under controlled climatic conditions is presented in **Figure 17**. Different temperature patterns (increase, decrease or stability) of accumulation of this flavonol in different cultivars emphasize the importance of examination and description of specific cultivar reactions rather than for the species in general. Especially, cultivars originating from breeding programs located at distant geographical locations with distinct breeding goals may have remarkably contrasting reaction patterns.

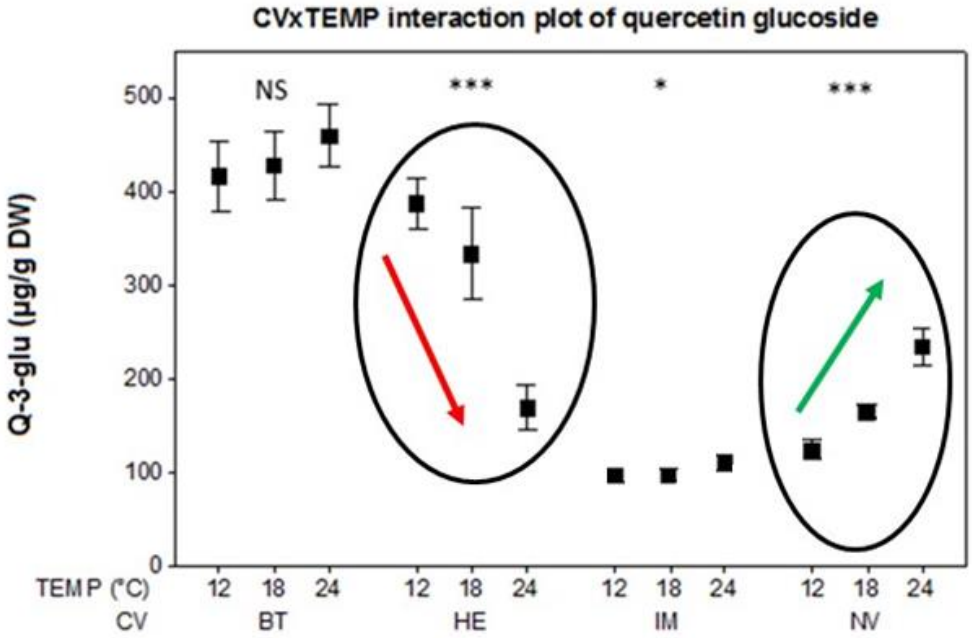


Figure 17. Example of cultivar x temperature interaction in the accumulation of quercetin glucoside in four black currant cultivars. Abbreviations used: BT ('Ben Tron'), HE ('Hedda'), IM ('Imandra'), NV ('Narve Viking'). Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (adapted from Paper IV)

Different accumulation of plant secondary metabolites in cultivars is due to genetic variants with DNA polymorphisms controlling the environmental responses. They can act by changing amino acid sequence causing alterations in protein function or affecting gene expression through altering of regulatory elements (cis-regulation), or alternatively, by affecting interacting transcription factors (trans-regulation) (Ehrenreich and Pfennig, 2015). Transcription factors (TFs) are proteins that bind to DNA and regulate gene expression by altering transcription levels. In tomato, the HD-Zip I family transcription factor modulates ascorbate accumulation by positively regulating the L-galactose pathway through binding to the promoter of an ascorbic acid biosynthetic gene encoding GDP-D-mannose pyrophosphorylase 3 (SlGMP3), as well as GDP-Man-3′,5′-epimerase 2 (SlGME2), GDP-L-Gal phosphorylase (SlGGP) and SlGMP4 (Hu et al., 2015). Differences in expression of genes from the flavonoid biosynthesis pathway are controlled by interaction between the DNA binding MYB10, bHLH33, and WD40 transcription factors (Yang et al., 2015). In addition, environmental conditions may change the expression of the bHLH transcription factor and control anthocyanin accumulation by regulating transcript levels of anthocyanin biosynthetic genes (Qiu et al., 2016). The anthocyanin biosynthetic pathway has been shown to be highly conserved across plant taxa (Petroni and Tonelli, 2011). It may therefore be

presumed that the mechanism that has been described for tomato and other crop plant species, may also be operative in black currants. Expression of various TFs can be controlled by epigenetic modifications (DNA methylation and histone modifications) which can also be affected by the environment (Roy, 2015). In addition, Chen et al. (2015) observed a positive correlation between a number of transcription factors and lower phenotypic plasticity (narrower range of reaction norm).

Better understanding of molecular and climatic regulation mechanisms responsible for cultivar differences in accumulation of secondary metabolites may help to develop black currant cultivars with wide adaptation to different climate, high nutritional value and predictable quality across distant production sites.

6. Main conclusions and future perspectives

Climatic conditions during the ripening of black currants had strong influence on berry chemical composition. The obtained results contribute to a deeper understanding of the complex relation between climate environmental conditions and black currant quality.

In general, cool summer conditions with ample precipitation resulted in high yields of berries with high nutritional value for all investigated black currant cultivars, and in general, these results were confirmed by experiments in controlled environments. The concentrations of a wide range of identified compounds were strongly influenced by temperatures over the 12-24 °C range. Daylength had only minor impacts on accumulation of health related phytochemicals. On the other hand, increased daily light integral under natural daylength conditions in the phytotron stimulated the accumulation of total monomeric anthocyanins (TMA) (Paper II). The same response was observed under increased radiation (PAR) in the field (Paper I). In general, low ripening temperature and higher daily light integrals lead to black currant berries with high nutritional value and better taste. However, under controlled climatic conditions only the influence of constant temperatures was investigated. Therefore, studies with black currant grown under fluctuating day/night temperatures are also required.

Plant genetic background is the main source of variation in fruit quality and has supreme influence on accumulation of bioactive compounds in black currant berries. Cultivars with high berry concentrations of health related compounds will be valuable progenitors for future black

currant breeding. Moreover, different stability in accumulation of various secondary metabolites by cultivars of different origins, gives a unique perspective for breeding of new cultivars with stable high quality. An example of a cultivar that combines those properties is ‘Imandra’, which had both the highest and the most stable concentrations of ascorbic acid. A deeper understanding of such issues requires detailed analysis at both metabolomic and transcriptomic levels to investigate the underlying molecular mechanisms responsible for such traits.

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Paper I

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ORIGINAL ARTICLE

Yield and fruit quality of black currant (*Ribes nigrum* L.) are favoured by precipitation and cool summer conditions

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The aim of this study was to examine the genetic and climatic impact on yield parameters and fruit chemical composition of black currant cultivars (*Ribes nigrum* L.). Correlation analysis between fruit parameters and climatic conditions over a period of eight years revealed a positive correlation between yield and precipitation during fruit development, whereas summer temperatures and radiation were negatively correlated with yield. Higher soluble solid concentrations occurred in years with high summer temperatures and radiation, while anthocyanins concentrations were negatively correlated with summer temperature. Furthermore, a negative correlation between phenolic compounds and radiation in June and July was observed. Temperature and radiation in late spring and summer were also negatively correlated with ascorbic acid concentration, while precipitation during summer was highly positively correlated with this important vitamin. These results indicate that to achieve high yield of quality black currants, rich in phenolic compounds, anthocyanins and ascorbic acid, cool summer conditions with ample precipitation are desirable. The observed cultivar variation in the content of health-related phytochemicals provides a good potential for further breeding of new cultivars with improved fruit quality.

Keywords: anthocyanins; ascorbic acid; black currant; climatic conditions; fruit quality; total phenolics

Introduction

Black currant (*Ribes nigrum* L.) is a perennial small shrub, indigenous to central and northern Europe, Caucasus, Central Siberia and Himalaya. It is commonly grown in Eastern, Central and Northern Europe, in addition to New Zealand and China, as a garden shrub and as an important commercial crop. Black currant fruits are known for their high content of health-related constituents with nutritive and potential medicinal properties (Häkkinen et al. 1999; Gopalan et al. 2012; Djordjevic et al. 2013). Because of the potential health benefits and good taste, black currant fruits are consumed fresh, but are also used for processing of jams, juice, jelly, syrup, wine and liqueur (Hummer & Barney 2002; Lim 2012). The berries are rich in anthocyanins, which act as food colourants and are polyphenols, known for their antioxidant properties (Bridle & Timberlake 1997; Gawel 1998). Black currant fruits are also known for their high concentration of vitamin C (L-ascorbic acid), which is an essential vitamin for humans. Vitamin C is a strong antioxidant, and has been used for prevention, and even cure of diseases (Padayatty et al. 2003). Ascorbic acid concentration is higher in black currants than in most other fruits and it also seems to be relatively stable in black currants, probably due to the presence of high amount of phenolic compounds and anthocyanins, which acts as protective agents of ascorbic acid (Miller & Rice-Evans 1997). These fruit quality components are strongly influenced by genotype (Lee & Kader 2000; Remberg et al. 2007; Figueiredo et al. 2008), but can also be significantly modified by environ-

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mental conditions (Lee & Kader 2000; Remberg et al. 2007; Figueiredo et al. 2008; Zheng et al. 2009; Walker et al. 2010; Krüger et al. 2011), and agronomic practices (Lee & Kader 2000). Another important fruit quality attribute is fruit size, which vary with genetic background and yield, as well as climate conditions (Hummer & Barney 2002). It is important to assess these quality characteristics and to understand how they are influenced by genetic background and climatic conditions. Here, the impact of climate on yield performance and fruit chemical composition of four black currant cultivars was studied over an eight-year period.

Materials and methods

Plant material and cultivation

The experiment was carried out during the years 2005–2012 on cultivars representing different genetic background, including the Scottish cultivars 'Ben Hope' and 'Ben Tron' and the Norwegian cultivars 'Kristin' and 'Varde Viking'. Plants were grown in a randomized field trial with three replicates of each cultivar at the Norwegian University of Life Sciences at Ås, Norway (59°39′N-10°45′E). The site was a south-facing slope with well-drained moraine soil. No irrigation or plant protection sprayings were applied, and pruning and fertilization was performed according to standard recommendations. The bushes were planted in 2003. First harvest took place when the plants were four years old. Berries of each cultivar were hand harvested when regarded as fully ripe. Samples (three replicates representing three bushes from each cultivar) were frozen within 2 h after harvest and stored in plastic boxes at –20°C until analysed.

Climatic data

Climatic data were collected from the meteorological station at the Norwegian University of Life Sciences. Data sets for the climatic conditions in the years 2005–2012 were used in this study. All climatic data are available at the Norwegian University of Life Sciences website (NMBU 2014).

Climatic variables are described by the sums of individual meteorological parameters. With respect to temperature, sums of daily mean temperatures for the periods 5, 10 and 15 days before harvest were recorded, as well as the sums of daily mean temperatures in May, June and July in addition to the sums of two months (May to June and June to July). The following abbreviations were used for the respective data periods: T 5, T 10, T 15, TMay, TJun, TJul, TMayJun, TJunJul. Photosynthetically Active Radiation (PAR; mol m^{-2}) sums for 5, 10 and 15 days before harvest, sum of PAR from May, June and July and sum of June to July were also recorded. PAR 5, PAR 10, PAR 15, PARMay, PARJun, PARJul and PARJunJul, respectively, were used, as abbreviations. UV radiation during 5, 10 and 15 days before harvest in addition to sum of UV radiation in July were also recorded and abbreviated as: UV 5, UV 10, UV 15, UV Jul, respectively. Precipitation during the months of May, June and July and from June and July together were recorded and abbreviated as: PREMay, PREJun, PREJul, PREJunJul. The climatic data were correlated with the various fruit quality parameters for assessment of statistical relationships.

Soluble solids, pH, titratable acidity and dry matter

For determination of soluble solids (SS), pH and titratable acidity (TA), berries (30 g) were homogenized using a blender (Braun MR400, Karlsruhe, Germany), followed by heating in a water bath at 50°C for 1 h with 10 µl pectinase (Pectinex Ultra SP-L, Novozymes, Bagsværd, Denmark). It was then filtered (Whatman 125 mm, Schleicher & Schuell, Dassel, Germany) and centrifuged at 400 rpm for 15 min (Eppendorf 5810 R, Hamburg, Germany) to obtain juice. The juice was used for determination of SS (Atago Palette PR-100, Tokyo, Japan), pH (Methrom 691 pH Meter, Herisau, Switzerland) and TA (Methrom 716 DMS Titrino and 730 Sample Changer, Herisau, Switzerland). For determination of dry matter (DM) content, berry homogenate (6–7 g) was dried at 100°C for 24 h in a drying oven (Termaks, Bergen, Norway) and stabilized in a desiccator before weighing.

Antioxidant capacity, total monomeric anthocyanins and total phenolic compounds

For analyses of antioxidant capacity (AOC, determined as Ferric reducing ability of plasma, the FRAP assay), total monomeric anthocyanins (TMA), and total phenolic (TP) compounds, berries (30 g) were homogenized with a blender (Braun MR400, Karlsruhe, Germany) and 3 g of homogenate was extracted with 1 mM HCl (37%) in methanol (30 mL). The samples (30 mL) were flushed with nitrogen, capped and vortexed (Vortex-T Genie 2, Scientific Industries Inc., Bohemia, NY, USA), followed by sonication at 0°C for 15 min in an ultrasonic bath (Bandelin SONOREX RK 100, Bandelin Electronic GmbH & Co., Berlin, Germany). The 30 mL samples were stored at –20°C until analysed. Prior to analysis, the samples were poured into a 2 mL micro tube (Sarstedt, Nürnbrecht,

Germany) and centrifuged at 13200 rpm for 2 min at 4°C (Eppendorf 5415 R, Hamburg, Germany). For analyses of AOC, TMA and TP a KoneLab 30i (Thermo Electron Corp., Vantaa, Finland) analyser was used. The AOC was determined by the FRAP assay as described by Benzie and Strain (1996). TMA was performed by the pH differential method based on the spectral characteristics of anthocyanins (Giusti & Wrolstad 2005), and TP was determined using the Folin–Ciocalteu method (Singleton et al. 1999). Results are reported as μmol Fe^{2+} per g of fresh weight (AOC), g cyanidin-3-glucoside equivalents (CGE) per kg of fresh weight (TMA) and g gallic acid equivalents (GAE) per kg of fresh weight (TP).

Ascorbic acid

For analyses of L-ascorbic acid (vitamin C, AsA), 25 g of frozen fruits were added up to 150 g with 1% (w/v) of oxalic acid, homogenized for 1 min and filtered (B 1/2, folded, Schleicher & Schuell, Dassel, Germany). Further, the resulting extract was passed through an activated Sep-Pak C18 cartridge (Waters Corp., Milford, MA, USA) and filtered through a 0.45 μm Millex HA filter (Millipore, Molsheim, France). Samples for AsA analyses were prepared as described by Wold et al. (2004) and analysed by HPLC as described by Williams et al. (1973) using an Agilent Technologies 1100 Series HPLC system (Waldbronn, Germany) comprising a quaternary pump, an inline degasser, an autosampler, a column oven and a ultraviolet (UV) light detector. The HPLC operation used Chemstation software (Agilent, Waldbron, Germany). Separation was achieved using a 4.6 mm × 250 mm Zorbax SB-C18 5 Micron column (Agilent Technologies, Palo Alto, CA, USA). The injection volume was set to 5 μL and isocratic elution was performed with 0.05 M KH_2PO_4 as mobile phase at 1 mL min^{-1} and 25°C. Detection of AsA was performed at 254 nm and quantified against calibration curves of freshly prepared standard solutions.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed to test the effects of cultivars, years and their interactions. Means were compared by Tukey's multiple comparison test. Principal Component Analysis (PCA) and Pearson's correlation coefficients were used to test the relations between climatic variables and fruit quality parameters. Data for TMA and TP for 2005 and 2006 were not recorded. The analyses were performed using MiniTab statistical software (16.1. MiniTab, MiniTab Inc., PA, USA). Since the majority of parameters showed statistically significant cultivar × year interactions (Table 1), correlations regarding individual cultivars are justified. Interpretation of correlation coefficient (Taylor 1990) is as follows: weak correlation: $r \leq 0.35$, moderate correlation: $r = 0.36$–0.67, high correlation: $r = 0.67$–0.90, very high correlation: $r = 0.91$–1.0. The most significant correlation trends are marked by circles in the figures.

Results and discussion

Table 1 demonstrates differences between both cultivars and years for nearly all measured physicochemical parameters given on a fresh weight basis. The only exception was TA which did not vary between years. Significant cultivar × year interactions were revealed for most parameters, indicating that the cultivars respond differently to the particular climatic conditions in the various years. PCA was applied to visualize the general relationships between individual physicochemical parameters representing all cultivars and selected weather variables (TMay, TJun, TJul, TMayJun, TJunJul, PARMay, PARJun, PARJul, PARJunJul, PREMay, PREJun, PREJul, PREJunJul). The first two components of the PCA plot are shown in Figure 1. Quality parameters that are close to the climatic variables denote strong positive correlations, while the strong negative correlations are denoted by parameters that are more symmetrically distant on the PCA plot area.

The first two principal components (PCs) are responsible for 30.0% and 18.6% of variance, respectively, with a total variance 48.6%. The PC 1 is mainly attributed by TP (loading: 0.234) and yield (0.204) together with its influencing factors, PAR and precipitation (highest: PAR JunJul and Pre JunJul, with loadings –0.370 and 0.332, respectively). The highest loadings of PC 2 are observed for AOC (–0.339), berry weight (BW; –0.323), TMA (–0.311), TP (–0.248) and AsA (–0.238) with influencing parameters PREJul (0.371), TMayJun (0.368) and TJun (0.283). A third principal component (PC 3) explaining 14.3% of the variance is mainly dominated by pH, DM and AsA with loadings 0.334, –0.310 and –0.270, respectively. The highest loadings for influencing parameters were represented by TJunJul (–0.445) and TJun (–0.310; data not shown).

To investigate relations between the climatic conditions and fruit quality parameters of individual black currant cultivars in more details, figures summarizing the Pearson's correlation coefficients are presented and discussed below.

Positive correlation was observed between yield and precipitation (Figure 2). Therefore, dilution effects might be assumed for AOC, TMA, TP and

Table 1. Yield and quality components of black currant fruits determined in four cultivars over a period of eight years[a].

		Yield (kg)	BW (g)	DM (%)	SS (%)	TA (%)	pH	AOC (μmol g^{-1})	TMA (g CGE kg^{-1})	TP (g GAE kg^{-1})	AsA (g kg^{-1})
Cultivar[b]	'Ben Hope'	8.29 ± 2.81 ab	0.96 ± 0.31 ab	18.54 ± 0.99 a	16.99 ± 1.33 a	4.48 ± 0.28 a	2.79 ± 0.056 d	109.25 ± 24.10 ab	2.74 ± 0.41 b	7.09 ± 0.68 a	2.01 ± 0.1 a
	'Ben Tron'	9.22 ± 2.80 a	1.01 ± 0.17 a	17.21 ± 0.90 c	15.09 ± 0.89 c	3.27 ± 0.09 c	2.92 ± 0.07 b	105.31 ± 15.59 b	2.50 ± 0.29 c	6.99 ± 0.66 a	1.67 ± 0.1 b
	'Kristin'	7.70 ± 2.81 b	0.84 ± 0.16 b	17.73 ± 0.68 b	15.40 ± 1.23 c	3.06 ± 0.12 d	3.05 ± 0.06 a	81.49 ± 13.24 c	2.19 ± 0.38 d	5.41 ± 0.78 c	1.02 ± 0.14 c
	'Varde Viking'	7.70 ± 2.92 b	0.97 ± 0.19 ab	18.17 ± 1.02 a	16.52 ± 1.03 b	4.34 ± 0.18 b	2.88 ± 0.05 c	111.43 ± 20.80 a	2.97 ± 0.32 a	6.55 ± 0.65 b	1.67 ± 0.21 b
Year[c]	2005	7.60 ± 1.48 bc	1.10 ± 0.22 a	17.16 ± 0.75 d	15.33 ± 0.69 c	3.80 ± 0.64 a	2.83 ± 0.10 e	68.86 ± 9.41 e	nm	nm	1.47 ± 0.37 c
	2006	7.05 ± 2.34 c	0.77 ± 0.45 b	19.00 ± 0.97 a	17.27 ± 1.16 a	3.81 ± 0.77 a	2.92 ± 0.11 bc	117.93 ± 17.76 ab	nm	nm	1.55 ± 0.40 bc
	2007	9.61 ± 2.45 ab	0.88 ± 0.11 ab	17.62 ± 1.12 bcd	15.29 ± 1.96 c	3.66 ± 0.67 a	2.89 ± 0.07 cd	94.17 ± 15.63 d	2.45 ± 0.50 b	7.13 ± 0.71 a	1.67 ± 0.38 ab
	2008	3.12 ± 1.13 d	0.89 ± 0.15 ab	18.08 ± 0.97 bc	16.58 ± 1.38 b	3.87 ± 0.74 a	3.00 ± 0.11 a	104.50 ± 14.86 c	2.66 ± 0.41 b	6.17 ± 0.88 b	1.57 ± 0.53 bc
	2009	9.77 ± 2.13 a	0.90 ± 0.10 ab	17.57 ± 0.67 cd	15.42 ± 0.92 c	3.73 ± 0.57 a	2.95 ± 0.10 b	94.09 ± 13.56 d	2.23 ± 0.37 c	5.66 ± 0.95 c	1.48 ± 0.36 c
	2010	9.01 ± 1.63 ab	0.92 ± 0.07 ab	18.30 ± 0.79 ab	15.66 ± 1.20 c	3.83 ± 0.63 a	2.94 ± 0.08 b	110.92 ± 13.77 bc	2.59 ± 0.35 b	7.08 ± 0.91 a	1.64 ± 0.41 ab
	2011	10.60 ± 1.61 a	1.08 ± 0.14 a	17.52 ± 0.75 cd	15.66 ± 0.84 c	3.80 ± 0.65 a	2.88 ± 0.12 d	104.46 ± 9.82 c	2.63 ± 0.43 b	6.56 ± 0.64 b	1.63 ± 0.34 ab
	2012	7.94 ± 1.69 abc	1.04 ± 0.06 a	18.04 ± 1.10 bc	16.79 ± 1.01 ab	3.79 ± 0.74 a	2.87 ± 0.09 d	120.05 ± 28.97 a	3.04 ± 0.24 a	6.45 ± 0.86 b	1.74 ± 0.41 a
ANOVA results with MS	Cultivar (A)	10.15***	0.14**	7.93***	19.48***	12.58***	0.26***	4583.1***	1.98***	10.61***	4.17***
	Year (B)	74.98***	0.16***	3.97***	7.02***	0.05 NS	0.04***	3246.1***	0.86***	3.71***	0.11***
	A × B	10.15***	0.05 NS	1.39***	2.58***	0.04 NS	0.001*	373.0***	0.19***	0.46**	0.05***
	Error	2.130	0.032	0.299	0.232	0.029	0,0006	37.10	0.028	0.14	0.013

[a]All values are given on a fresh weight basis. Data are means ± SD of three bushes of each cultivar across all years; [b]All cultivars in each year; [c]Means that do not share a letter are different at $p < 0.05$ level, with comparisons performed using Tukey's test.MS, mean squares; nm, not measured. BW, berry weight; DM, dry matter; SS, soluble solids; TA, titratable acidity; AOC, antioxidant capacity; TMA, total monomeric anthocyanins; TP, total phenolics; AsA, ascorbic acid; NS, not significant. *Significance level:* $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$.

AsA concentrations as suggested likewise by the data for DM and SS in Figure 3. To avoid such dilution-biases for these parameters, correlations were performed on a dry weight basis, which increased the strength of the analysis (Remberg et al. 2010).

Yield and BW

Fruit yield (kg, in FW per bush) and average BW (in g) from three replicates of each cultivar were recorded. The results showed significant differences between both cultivars and years (Table 1). The highest yielding cultivar in this experiment was 'Ben Tron' with 9.2 kg, and the lowest was 'Kristin' with 7.7 kg. The highest yield was registered in 2011 with 10.6 kg (average of all cultivars) while the lowest yield was registered in 2008 (3.1 kg), thus showing great variation due to environmental conditions. The experimental set-up revealed correlations for yield, BW and climatic parameters (Figure 2). Positive correlation was shown between yield and precipitation during all investigated periods of the season. On the other hand, negative correlations were generally observed between yield and sums of PAR and UV during all periods of the season. In addition, a general negative relationship was noted between summer temperature and fruit yield, although with some variation between cultivars.

BW varied both between cultivars and years. 'Kristin' had the smallest berries (0.84 g) while 'Ben Tron' had the largest berries with 1.01 g (Table 1). In most cultivars, BW was negatively correlated with temperature during all periods of the growing season (Figure 2). The exception was 'Ben Hope' in which BW was positively correlated with

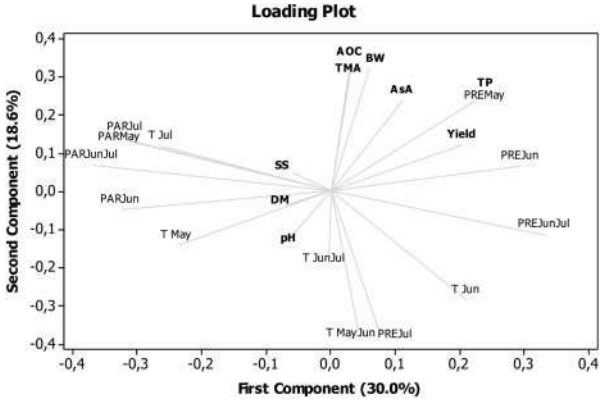

Figure 1. PCA loading plot showing the position of fruit physicochemical parameters (BW, berry weight; DM, dry matter; SS, soluble solids; TA, titratable acidity; AOC, antioxidant capacity; TMA, total monomeric anthocyanins; TP, total phenolics; AsA, ascorbic acid) for all black currant cultivars in relation to selected weather variables (T, temperature; PAR, photosythetically active radiation; PRE, precipitation).

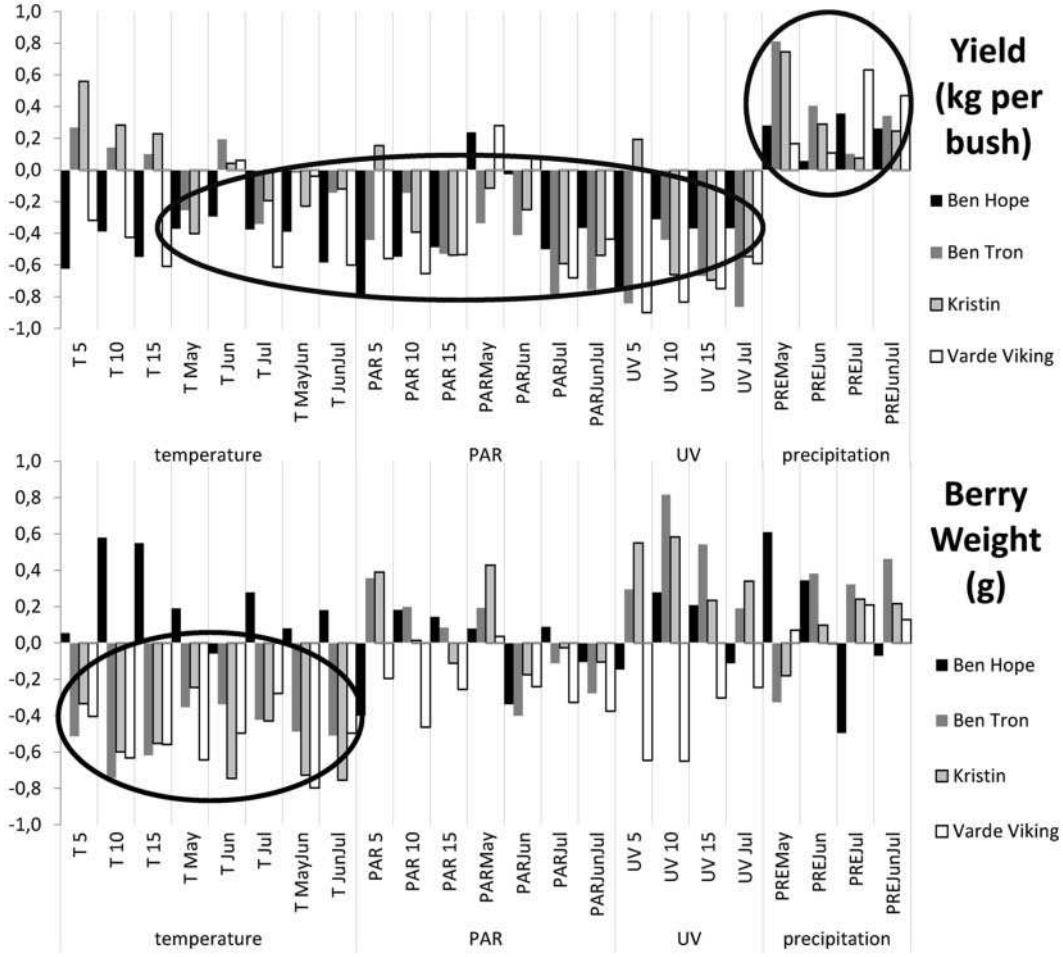

Figure 2. Pearson's *r* correlation coefficients between weather variables (temperature, PAR, UV radiation, precipitation) and the fruit crop attributes: yield and BW.

temperature during fruit ripening (T 10 and T 15). Otherwise, correlations between BW and climatic conditions varied, and no clear trends were observed. This could be due to the fact, that yield and BW are highly influenced not only by climatic conditions during fruit development, but also by other factors throughout different periods of the year (winter chilling, frost damage, pollination, flower drop, etc.; Kahu et al. 2009; Kikas et al. 2011).

Dry matter

DM concentrations in berries varied significantly between years and among cultivars (Table 1). Positive correlations were observed regarding all investigated sums of temperatures. The highest positive correlation was observed for temperature, especially sums of 5, 10 and 15 days before harvest. Also PAR and UV radiation were mainly positively correlated with DM. DM concentration in fruits was lower in seasons with high precipitation during the summer (Figure 3). These results were similar to those described by Giné Bordonaba and Terry (2010) in strawberries (*Fragaria* × *ananassa*), where controlled water deficit irrigation reduced DM content in the fruits.

Soluble solids

SS concentration varied between both years and cultivars (Table 1). The highest SS concentrations were observed in 2006 (17.3%), and the lowest in 2007, with a level of 15.3%. Significant variations were observed between the cultivars, with mean concentrations of 15.1% in 'Ben Tron' and 17.0% in 'Ben Hope'. As with fruit yield, SS concentration was positively correlated with temperature in addition to sums of PAR and UV before harvest, and negatively with precipitation (Figure 3). These results are in agreement with previous observations, where positive correlations between temperatures in July and SS were reported for black currants (Zheng et al. 2009; Kaldmäe et al. 2013).

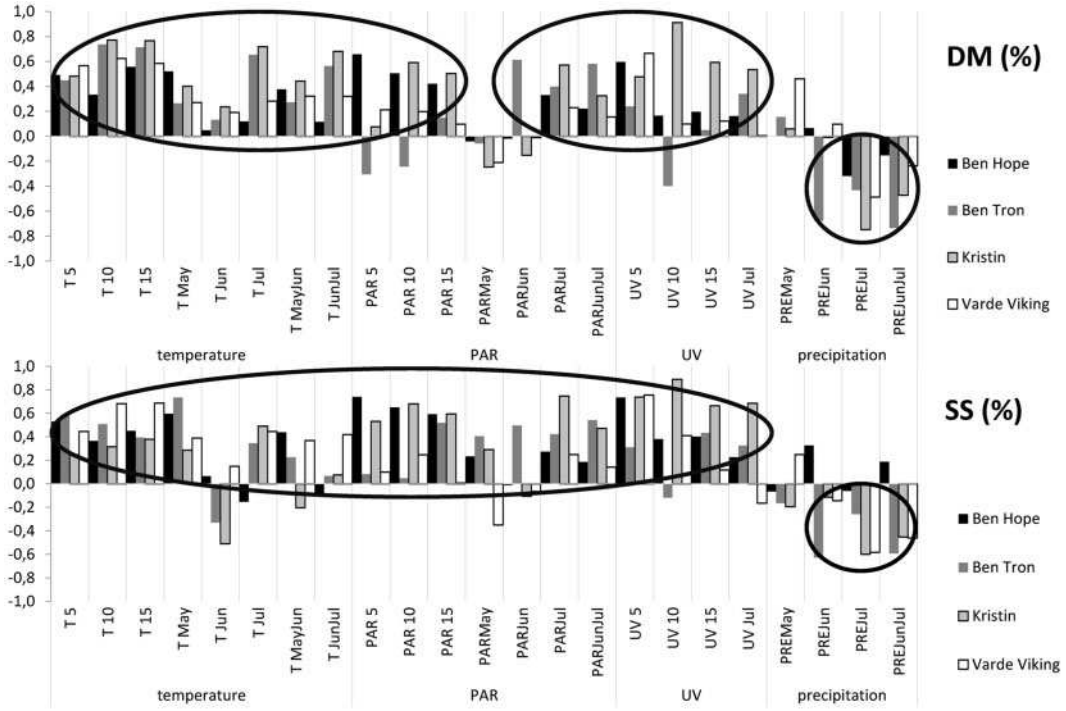

Figure 3. Pearson's *r* correlation coefficients between weather variables (temperature, PAR, UV radiation, precipitation) and the fruit quality attributes (DM and SS).

Titratable acidity

TA varied significantly between cultivars (Table 1). 'Kristin' had the lowest TA with 3.1% as average over eight years. The highest value was observed in 'Ben Hope' with an average content of 4.5%. TA seemed not to be affected by climatic conditions and in consequence was not correlated with the prevailing climatic conditions during the investigated period.

pH

Fruit pH values differed significantly between both years and cultivars. The highest pH was measured in 'Kristin' (3.05) and the lowest in 'Ben Hope' (2.79; Table 1). The pH tended to increase (less acidic) with increasing temperatures and higher light intensity. On the one hand, it was positively correlated with the sums of temperatures in May and June, temperatures of two months periods (May to June and June to July) in addition to the sums of PAR and UV (Figure 4). The same response to elevated temperature was observed in grapes (*Vitis vinifera*)under controlled temperature conditions (Jackson 1986). On the other hand, pH was negatively correlated with precipitation in May and June. The relationship shifted to positive with progress of the season.

Antioxidant capacity

AOC in black currant fruits varied between both cultivars and years (Table 1). In 2005, the average

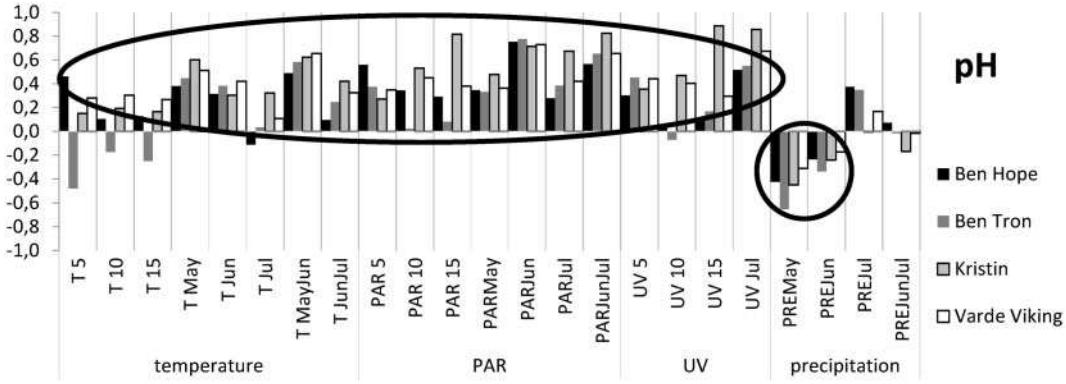

Figure 4. Pearson's *r* correlation coefficients between weather variables (temperature, PAR, UV radiation, precipitation) and fruit pH.

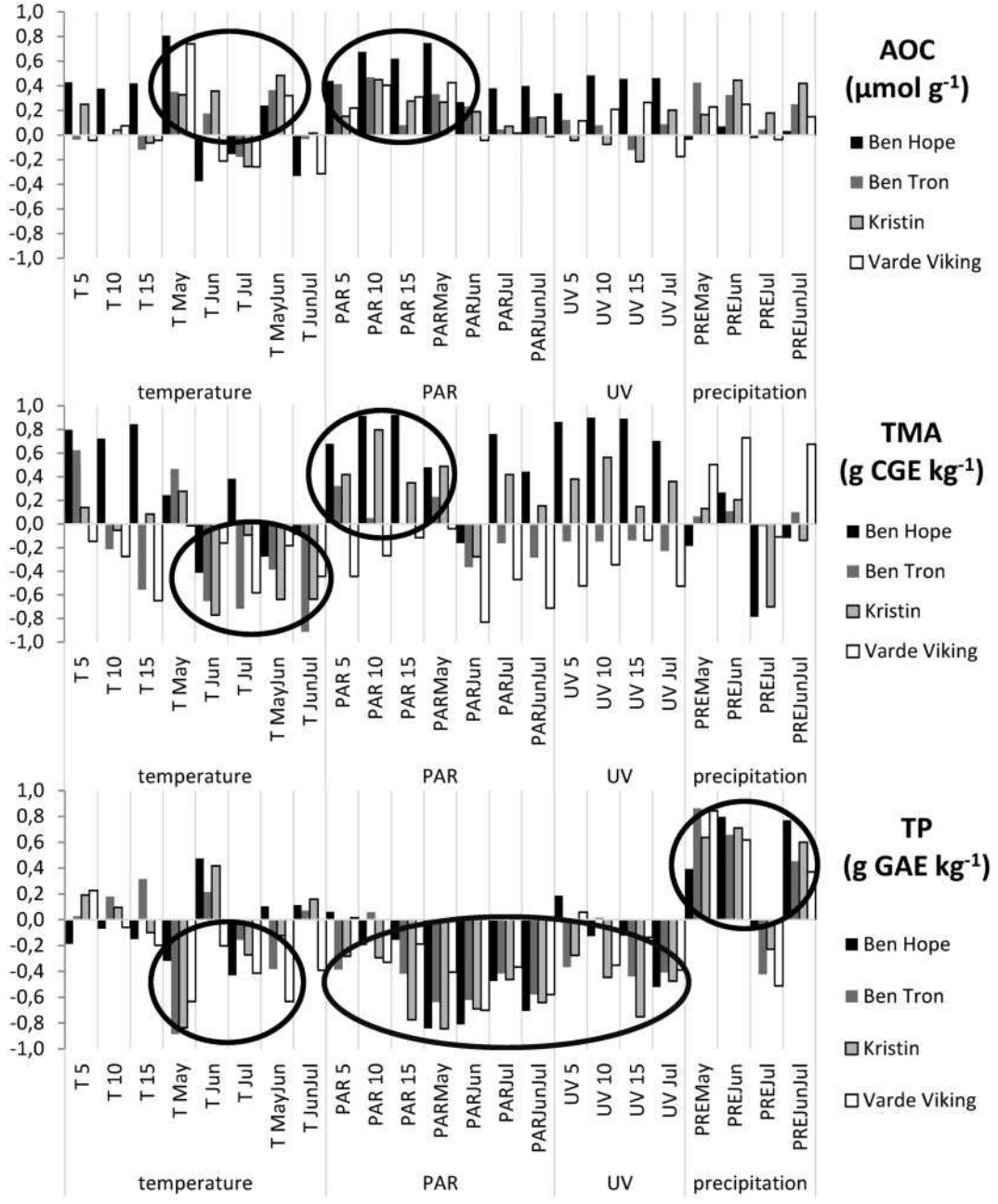

Figure 5. Pearson's *r* correlation coefficients between weather variables (temperature, PAR, UV radiation, precipitation) and antioxidant capacity (AOC), total monomeric anthocyanins (TMA), total phenolic compounds (TP; DW basis).

AOC for all cultivars was 68.9 µmol g^{-1} FW, while in 2012 it was almost twice as high (120.1 µmol g^{-1} FW). Significant differences in AOC between investigated cultivars were also observed. 'Kristin' had the lowest value with 84.6 µmol g^{-1} FW, while 'Varde Viking' had the highest AOC with 111.4 µmol g^{-1} FW.

Previous investigations with strawberry showed higher antioxidant activity in berries grown at higher temperatures (30°C day/25°C night) than lower (18°C day/12°C night) temperature conditions, which was related to different flavonoid concentrations (Kalt et al. 2001). Moreover, correlation analysis performed by Remberg et al. (2012) indicated increased antioxidant activity in black currants in seasons with higher global radiation, higher temperature and lower precipitation, although these correlations were relatively weak. Our results confirm these previous observations mainly regarding the effects of PAR. The highest positive correlations were observed between PAR just before harvest. The effect of temperature is not consistent, showing the strongest positive relation in May and May to June (Figure 5).

Total monomeric anthocyanins

The concentration of TMA varied significantly between cultivars and years (Table 1). The highest concentration in black currant fruits was recorded in 2012 (3.04 g CGE kg^{-1} FW) and the lowest in 2009 (2.23 g CGE kg^{-1} FW). On average of all years, 'Kristin' was characterized by the lowest anthocyanin concentration (2.19 g CGE kg^{-1} FW) and 'Varde Viking' with the highest concentration of 2.97 g CGE kg^{-1} FW.

Anthocyanins are known to act as UV-protecting agents in plants (Bridle & Timberlake 1997). However, in this study TMA concentration showed opposite correlation with UV radiation in the cultivars 'Ben Hope' and 'Varde Viking'; the former showing a high positive correlation and the latter a negative correlation with UVradiation during fruit ripening (Figure 5). Except for 'Varde Viking', TMA was generally positively correlated with PAR during the last 5–15 days before harvest. In addition, a consistent negative correlation was observed between anthocyanin concentration and temperature in the late phase of fruit development (Figure 5). These results are similar to the findings of Saure (1990) and Ubi et al. (2006), who observed that lower temperature favoured anthocyanin biosynthesis in apple skin (*Malus domestica*). Low temperature is an inducing factor of the expression of the chalcone synthase, anthocyanidin synthase and UFGluT (UDP-glucose: flavonoid 3-O-glucosyltransferase) genes, which are the key genes controlling biosynthesis (Ubi et al. 2006). Also, Mori et al. (2005) observed that grapes grown under higher temperatures accumulated less anthocyanins, possibly due to negative influence of elevated temperature on enzyme activity as well as intermediates (e.g. anthocyanidin) stability. They suggest that both UFGluT (UDP-glucose: flavonoid 3-O-glucosyltransferase) and Phenylalanine ammonialyase activities are highly sensitive to temperature and are the most important factors in the regulation of anthocyanin accumulation.

High anthocyanin content in blue-black UV-reflecting fruits is an attraction factor for birds, which seem to recognize high anthocyanin concentration (positively influencing immune system in frugivorous birds) using the avian long-wave receptor (Schaefer et al. 2008). From an evolutionary perspective, anthocyanins act simultaneously as protecting agents and seed dispersers' attractors. Therefore, black currants do not tend to produce additive anthocyanins in response to stress induced by UV radiation because of their evolutionary-based inherently high anthocyanin concentrations (Schaefer et al. 2008).

Total phenolic compounds

Differences in TP concentration in the four black currant cultivars were observed (Table 1). Fruits from 'Kristin' contained on average 5.41 g GAE kg^{-1} FW, which was the lowest concentration recorded, while 'Ben Tron' was the cultivar with the highest concentration with an average of 7.09 g GAE kg^{-1} FW. Over the recording period, the lowest concentration of phenolic compounds was noted in 2009 (5.66 g GAE kg^{-1} FW) and the highest in 2007 (7.13 g GAE kg^{-1} FW).

Precipitation in May and June and sums of precipitation in June to July were positively correlated with TP concentration, while negative correlations were noted with precipitation in July. The TP concentration was not markedly affected by sum of temperatures during the summer months showing not consistent, but mainly negative correlations. On the other hand, PAR in May, June, July and in June to July showed moderate to high negative correlation with the concentration of TP compounds for all tested black currant cultivars (Figure 5). Effects of PAR were opposite to those reported by Savikin et al. (2013) from a field experiment in Serbia, where the concentration of phenolic compounds in black currants was significantly lower in berries grown under controlled shading conditions compared to full sun exposure. One reason for those contradictory results might be the contrasting climate conditions in Norway and Serbia. For plants grown in Serbia, where climatic conditions differ from the native habitat of black currants, high temperature and excessive radiation can be stressful and may cause accumulation of phenolic compounds acting as protecting agents due to this stress (Saure 1990). Nonetheless, results from Finland (Yang et al. 2013), with red and white currants (*Ribes rubrum*) confirm our observation that higher PAR and temperature during the ripening reduce the concentration of phenolic compounds in *Ribes* fruits.

Ascorbic acid

Ascorbic acid is an important quality component in black currant fruits. This study revealed large variations in AsA concentration among cultivars and years (Table 1). The mean AsA concentration across all black currant cultivars amounted to 1.59 g kg^{-1} FW. The highest concentration was observed in 2012 with 1.74 g kg^{-1} FW while the lowest average AsA concentration in all investigated fruits was observed in 2005 (1.47 g kg^{-1} FW). The highest concentration of AsA was recorded in 'Ben Hope' with 2.01 g kg^{-1} FW, followed by 'Ben Tron'

and 'Varde Viking' with 1.67 g kg^{-1} FW and 'Kristin' which had the lowest concentration with 1.02 g kg^{-1} FW.

Temperature in July, sum of temperatures in May and June and sum of temperature in June to July showed negative correlations with AsA concentration in all investigated cultivars. Negative correlations with sum of temperatures during 10 and 15 days before harvest were also observed. Summer precipitation (sum of June and July) was highly positively correlated with AsA concentration in fruits of all cultivars (Figure 6). PAR in June, July and June to July was mainly negatively correlated with AsA concentration. Sum of PAR in May and shortly before harvest (5, 10 and 15 days) was positively correlated with AsA concentration in 'Varde Viking' and 'Ben Hope' but not in the other cultivars. The observed decline of AsA concentration during warmer growing seasons is in agreement with a previous study in controlled climate, where the 'Frost Satsuma' mandarins (*Citrus unshiu*) grown under cooler temperatures contained more vitamin C than those from warmer conditions (Nagy 1980). Moreover, in an experiment with tomatoes (*Lycopersicon esculentum*), growth temperature was negatively correlated with vitamin C concentration (Riga et al. 2008). A previous experiment with black currants under controlled environmental conditions also indicated that lower growth temperature may have some positive influence on vitamin C concentration (Redalen 1993). Likewise, lower temperature conditions (18°C day/12°C night) also promoted AsA accumulation in strawberries (Wang & Camp 2000). On the contrary, a correlation analysis from Scotland showed mainly positive effects of higher temperatures from April to June on vitamin C concentration in black currants (Walker et al. 2010). Negative impact of drought stress on vitamin C accumulation in cherry tomatoes (*Lycopersicon esculentum* var. *cerasiforme*) was demonstrated by De Pascale et al. (2007) and our results are in agreement with this finding. In a previous study, light intensity was mainly positively associated with AsA concentration in tomatoes (El-Gizawy et al. 1992). Experiment with highly reflective mulches gave non-conclusive results, enhancing vitamin C content only in the strawberry cultivar 'Elsanta' but not in 'Flamenco' (Atkinson et al. 2006). This is in agreement with our studies, where responses to the various environmental factors occasionally deviated considerably between cultivars, to the extent that it precluded general conclusions (e.g. PAR: TMA in Figure 5 and AsA in Figure 6).

These results imply that the accumulation of ascorbic acid is a complex process, which also depends on genetic background. Post-transcriptional or post-translational control of vitamin C accumulation in black currant fruits is suggested due to absence of correlations between expression of genes encoding the key enzymes (GDP-mannose pyrophosphorylase, DP-mannose 3,5-epimerase, DP-L-galactose phosphorylase and L-galactose-L-phosphate phosphorylase) in the L-galactose pathway (AsA accumulation) and AsA concentration in fruits (Walker et al. 2010). Negative correlations observed between AsA and PAR and temperature on the other hand, in this experiment, may have been due to co-variation with other environmental factors, particularly water availability (Figure 6).

Ascorbic acid and phenolic compounds are strong antioxidants that often accumulate in plants as a protecting agent against environmental stress (Kalt et al. 2001). It was therefore somewhat surprising that the concentrations of these components (Figures 5 and 6) were not positively correlated with stressful environments such as high temperature and

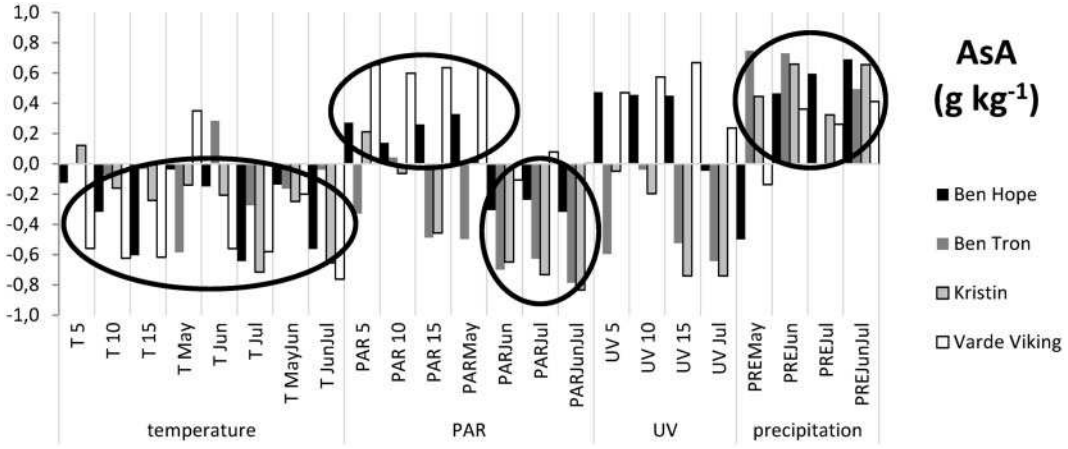

Figure 6. Pearson's *r* correlation coefficients between weather variables (temperature, PAR, UV radiation, precipitation) and vitamin C concentration (AsA; DW basis).

radiation, but rather to high precipitation. It should be kept in mind, however, that in its natural habitat, black currants is growing in moist areas in half-shade as an understory shrub (Lim 2012). It is therefore likely that the negative correlation observed between high temperature and radiation, and fruit yield and the content of important nutrient constituents such as vitamin C, as well as the opposite relationship with precipitation, are reflections of the species' adaptation to its natural habitat. It seems that even new black currant cultivars are poorly adapted to hot and dry weather. This may also be the explanation of the contrasting results of Vagiri et al. (2013) who reported that AsA was always lower in black currants grown at low temperature conditions in Northern Sweden than at high temperatures in the South. Possibly, the significantly higher rainfall at the southern location might have dominated over and masked the effect of temperature under those conditions.

In conclusion, for black currants grown under open field conditions in a Nordic climate, genotype was the main source of fruit variation in ascorbic acid, phenolic compounds, anthocyanins, AOC, DM, SS, pH, as well as yield and BW. Therefore, breeding of cultivars with improved fruit quality is an achievable goal. Moreover, our results indicate that climatic conditions are responsible for part of the variation. Investigation of stability and fitness of the cultivars together with the impact of the environmental conditions may help to predict, and to some extent, improve the fruit quality by proper selection of cultivars and cultivation sites. Because of the strong positive impact of precipitation on important phytochemicals, avoiding light soil conditions with low water storage capacity is suggested. The presented results showed, that yield and quality of black currant fruits decreased in warm and dry summers, confirming the low drought and heat tolerance, as a reflection of black currant evolutionary adaptation (Lim 2012).

Because of co-variation between several interacting factors in the natural environment, it is extremely difficult to sort out the main effects of the various climatic components in experiments under such conditions. For example, a negative correlation between PAR and fruit yield does not necessarily mean that high radiation *per se* is negative for yield, but rather that this condition usually is associated with high temperature and drought stress. Correlation analysis between fruit quality parameters and climatic conditions over the period of eight years showed that temperature, PAR, UV and precipitation can have a large impact on the accumulation of health-related phytochemicals in black currant fruits. Therefore, studies under controlled environment conditions are under way to better quantify these responses.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Paper II

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Effects of controlled post-flowering temperature and daylength on chemical composition of four black currant (*Ribes nigrum* L.) cultivars of contrasting origin

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ABSTRACT

The effects of post-flowering temperature and daylength on chemical composition of four black currant cultivars originating from distinct geographical locations have been studied under controlled environment conditions. Special emphasis was placed on establishing photoperiodic conditions that are not biased by simultaneous changes in daily light integral. Unexpectedly, berry ripening in terms of colour change was delayed by high temperature, apparently due to high temperature suppression of anthocyanin biosynthesis. The concentration of L-Ascorbic acid decreased with increasing temperature (12–24 °C), while the concentrations of total anthocyanins and total phenolics were at an optimum at 18 °C. Under identical daily light energy conditions (night interruption), photoperiod had no specific effect on the analysed fruit quality components, while natural long day conditions (with 9% additional daily light energy) lowered the pH and increased the concentration of total monomeric anthocyanins, and to a lesser extent, the concentration of soluble solids. The cultivars varied significantly in fruit chemical composition. The high-boreal cultivar 'Imandra' was the one least affected by environmental conditions. This study provides evidence that accumulation of ascorbic acid and total anthocyanins in black currant fruits is favoured by low post-flowering temperatures, while high daily light integrals also seem favourable for anthocyanin biosynthesis.

1. Introduction

Black currant (*Ribes nigrum* L.) is a perennial shrub, indigenous to central and northern Europe. It is an important commercial berry crop commonly grown in Europe, in addition to Asia, New Zealand and to a lesser extent, also in North America (Hummer and Barney, 2002).

Inclusion of berries in the human diet can provide numerous health benefits (Szajdek and Borowska, 2008; Paredes-López et al., 2010). There are convincing evidences that consumption of black currants can have positive effects on cardiovascular, nervous, ocular, skeletal and renal systems in the human body (Karjalainen et al., 2008; Gopalan et al., 2012). Black currants are an excellent source of ascorbic acid (AsA) and phenolic compounds such as anthocyanins as well as certain volatile chemicals (Heiberg et al., 1992; Brennan and Graham, 2009; Vagiri et al., 2013).

The quality and chemical composition of black currant are known to be influenced by both genetic and environmental factors, as well as cultivation practices, ripening stage and post-harvest treatment (Tabart et al., 2006; Zheng et al., 2009; Walker et al., 2010; Krüger et al., 2011; Vagiri et al., 2013). However, genotypes originating from distinct geographical regions and breeding programmes are the greatest source of variability in black currant chemical composition (Zurawicz et al., 1999; Krüger et al., 2011; Vagiri et al., 2013; Woznicki et al., 2015).

Despite of numerous research reports concerning environmental impact on quality and quantity of health related compounds in black currants, the existence of covariation of several climatic factors in the natural environment makes it exceedingly difficult to sort out the specific effects of the various environmental factors in field experiments (Krüger et al., 2011; Vagiri et al., 2013; Woznicki et al., 2015).

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Therefore, Redalen (1993) tried to simulate the impact of different European climates on black currant growth and quality in controlled environment experiments. However, only limited effects on the studied parameters were noticed. Wang and Zheng (2001) observed that increasing temperature in the 18/12 °C–25/22 °C day/night range enhanced the content of flavonoids and the antioxidant capacity in strawberries, while Remberg et al. (2010) showed that decreasing post-flowering temperature (12–24 °C) promoted the accumulation of ascorbic acid in raspberry. Mazur et al. (2014) also reported that long day photoperiodic treatment also enhanced health-beneficial components such as phenolic compounds and ascorbic acid in raspberry fruits.

In a recent paper (Woznicki et al., 2015), we analysed the relationship between summer climate and berry chemical composition of four black currant cultivars grown under field conditions in Southern Norway over an eight year period. An interesting finding was that berry concentrations of AsA and anthocyanins were negatively correlated with summer temperature, while precipitation showed the opposite relationship. However, because several climatic factors may change simultaneously in the natural environment (e.g. temperature, radiation, and precipitation), such co-variation makes it exceedingly difficult to correctly assess the impact of the individual factors. Therefore, we wanted to find out whether the same effects of temperature could be verified under controlled environment conditions. In the following, we present the results of such an experiment in which the effects of controlled post-flowering temperature and daylength on berry composition of four black currant cultivars of contrasting origin were studied.

Special emphasis was placed on establishing photoperiodic conditions that are not biased by simultaneous changes in daily light integral. Comparisons were also made with berries maturing under ambient, outdoor conditions.

2. Materials and methods

2.1. Plant material, cultivation and experimental design

Single-stemmed plants of four black currant (*R. nigrum* L.) cultivars of contrasting geographical origin were propagated from semi-softwood cuttings as described by Sønsteby and Heide (2011). The propagules originated from virus indexed stock plants. The cultivars were the high-boreal Russian cultivar 'Imandra', originating from the Kola Peninsula (67°30′N), 'Hedda' and 'Narve Viking' from the Norwegian breeding program at Ås (59°40′N) and 'Ben Tron' from the Scottish breeding program in Dundee (56°30′N). Pots filled with a coarse-texture sphagnum peat growth medium with a pH of 5.8 were used, and the plants were fed by automatic fertigation with a complete fertilizer solution throughout the whole experimental period as described by Sønsteby et al. (2009). The plants were raised in a greenhouse at 20 °C in 24 h long days (LD), until they had produced 12 or more nodes (leaves) and the height of ca. 45 cm. Then, on 16 July, they were moved outdoors for further growth and development under natural temperature and day-length conditions (59°40′N) until growth cessation in late November. The plants had then an average height of 140 cm, and 32 nodes (Fig. 1). After leaf abscission and hardening outdoors, the plants were moved into a cold store at 0 °C on 27 November for controlled over-wintering and breaking of bud dormancy.

On April 7, all plants were taken out of the cold store and placed under outdoor conditions at Ås (59°40′N). Temperature conditions during early growth and flowering as well as during the phytotron treatment period are shown in Fig. 2. The plants were covered with fleece for the first ten days to avoid desiccation, before they were placed in four east-west oriented rows (1.5 m between the rows, and a within-row spacing of 0.3 m), in a randomized block design.

Fig. 1. Appearance of the single stem plants at the end of the first growing season. From left: 'Imandra', 'Hedda', 'Ben Tron' 'Narve Viking' (photo taken 11 October 2013).

To keep the plants stable, they were tied to plastic coated wire trellis with plastic clips. The plants were automatically fertigated with a complete fertilizer solution with a 2:3 mixture of Superba™ Rød (9-5-25-4% NPKMg + micronutrients) and Calcinit™ (15.5–19% NCa) (Yara International, Oslo, Norway) with electric conductivity (EC) 1.0–1.2 mS cm^{-1} three times a day throughout the season.

After flowering and pollination, approximately three weeks before full ripeness (Fig. 3A), the plants were moved into the Ås phytotron (59°40′N, 10°45′E) and exposed to combinations of three temperatures (12, 18, 24 °C) and the following photoperiod conditions: (a) natural long summer day, 18–19 h (Natural LD), (b) 10 h summer daylight (SD) and (c) 10 h SD + 3 h night interruption in the middle of the 14 h dark period (SD + NI). The cultivars were moved sequentially into the phytotron based on observations of their earliness, on the dates as shown in Table 1. The daylight compartments were maintained at constant temperatures (±1 °C) and a water vapour pressure deficit of 530 Pa was maintained at all temperatures. All plants received 10 h of summer daylight from 08:00 h to 18:00 h in the daylight compartments. Whenever the photosyn-

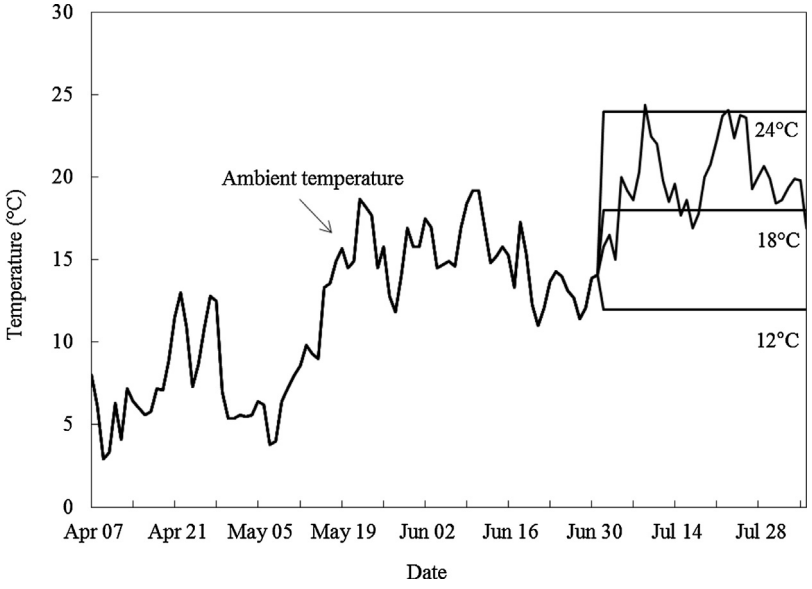

Fig. 2. Average daily mean outdoor (ambient) temperatures during the entire experimental period, and the constant temperatures in the phytotron compartments during cultivation there.

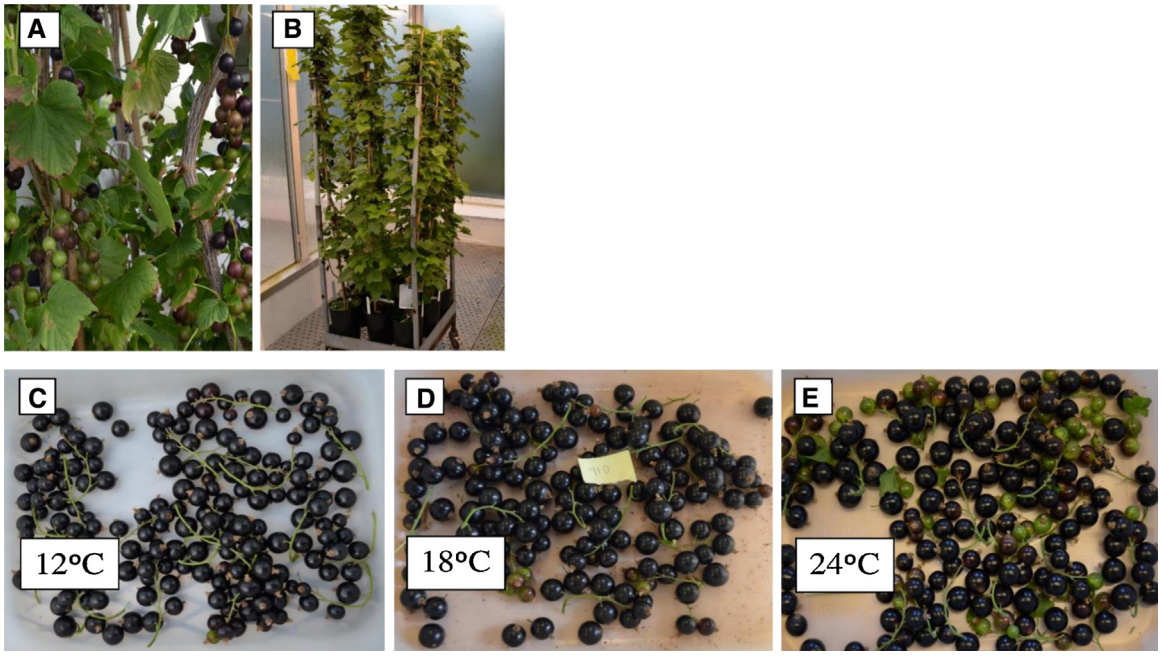

Fig. 3. (A) Berry ripening stage at the time when the plants were moved into the phytotron. (B) A trolley with two plants of each cultivar representing one of four treatment replications. (C) 'Hedda' harvested on day 22 at 12 °C, (D) 18 °C and (E) 24 °C.

thetic photon flux density (PPFD) in the daylight rooms fell below 150 μmol quanta m^{-2} s^{-1} during this 10-h period, an additional 125 μmol quanta m^{-2} s^{-1} was automatically added, using Philips HPT-I 400 W lamps (Amsterdam, Netherlands). Then, in order to reduce the additional light energy effect of the night interruption treatment (b) to a minimum, and thus to avoid confounding effects of photoperiod and daily light energy (Thomas and Vince-Prue, 1997), low-intensity light (approx. 7 μmol quanta m^{-2} s^{-1}) from incandescent lamps (70 W) was used for this treatment. In this way, the additional light energy of the night interruption amounted to less than 0.5% of the total daily light integral. On the other hand, meteorological data from the weather station at the Norwegian University of Life Sciences revealed that the total daily light energy provided to the plants under natural daylength conditions in the phytotron was about 9% higher than in the other photoperiodic treatments (SD and SD + NI). As an untreated control, plants of all cultivars were also grown outdoors under ambient light and temperature conditions.

Each treatment combination had four replications, each comprising of two plants of each cultivar on separate trolleys (Fig. 3B), resulting in eight plants per treatment. To ensure equal light conditions to all plants, only the eight positions along the edges of the trolleys were occupied by the plants. The trolleys were randomly distributed in the daylight rooms by the every-day movements to and from the photoperiod treatment rooms. Groups of eight plants of each cultivar also remained under ambient outdoor conditions at temperatures as shown in Fig. 2. During the experimental period,

Table 1
Timing and duration of the controlled environment treatments. Start and end of treatment are the dates when the bushes were placed in and taken out of the growth chambers or paralelly grown outdoors (Ambient).

	'Imandra'			'Hedda'			'Ben Tron'			'Narve Viking'		
	Start of treatment	End of treatment	Total days of treatment	Start of treatment	End of treatment	Total days of treatment	Start of treatment	End of treatment	Total days of treatment	Start of treatment	End of treatment	Total days of treatment
12 °C	July 02	July 18	16	July 09	July 29	20	July 09	July 25	16	July 16	August 05	20
18 °C	July 02	July 22	20	July 09	July 31	22	July 09	July 28	19	July 16	August 07	22
24 °C	July 02	July 25	23	July 09	August 01	23	July 09	July 31	22	July 16	August 08	23
Ambient	July 02	July 21	19	July 09	July 30	21	July 09	July 27	18	July 16	August 06	21

the outdoor daily mean temperature varied between 18 and 24 °C. No precipitation was recorded during this period.

Harvesting dates are presented in Table 1. In all treatments, berries were harvested when fully ripe, based on berry softness and visual assessment of berry colour. Berries were hand harvested in polyethylene boxes, and immediately frozen at −20 °C until analysed. The results from the quality analyses are presented on a dry weight basis, to avoid any possible dilution bias (cf. Remberg et al., 2010).

2.2. Chemical analyses

For determination of dry matter content (DM), 6–7 g of berry homogenate were dried at 100 °C for 24 h in a drying oven (Termaks, Bergen, Norway) and stabilized in a desiccator before weighing. For determination of soluble solids (SS), titratable acidity (TA), pH and optical density (OD), berries (30 g) were homogenized with a blender (Braun MR400, Karlsruhe, Germany), heated in a water bath at 50 °C for 1 h with 10 μl pectinase (Pectinex Ultra SP-L, Novozymes, Bagsværd, Denmark), filtered (Whatman 125 mm, Schleicher & Schuell, Dassel, Germany) and centrifuged at 400 rpm for 15 min (Eppendorf 5810 R, Hamburg, Germany) to obtain juice. The juice was used for determination of SS (Atago Palette PR-100, Tokyo, Japan), TA (Methrom 716 DMS Titrino and 730 Sample Changer, Herisau, Switzerland) and pH (Methrom 691 pH Meter, Herisau, Switzerland). Prior to the OD analyses, the juice was diluted to a 1% solution with distilled water. OD was determined using a spectrophotometer (Shimadzu UV mini 1240, Kyobashi, Japan), and results are given as a ratio between absorbance at 520 and 410 nm.

L-Ascorbic acid (AsA) content was determined according to Aaby et al. (2007) with some modifications. Approximately 5 g of frozen berry homogenate was placed into a polypropylene tube containing 20 mL of ice-cold extraction solution (5% meta-phosphoric acid with 1 mM disodium-dihydrogen-EDTA). The extract was homogenized using a Polytron PT 3000 homogenizer (Kinematica AG, Luzern, Switzerland) for 30 s at 24.000 rpm. The homogenate was poured into a volumetric flask and the total volume adjusted to 50 mL with extraction solution. After filtration (Grade 595 1/2, Schleicher & Schüll GmbH, Dassel, Germany), the extract was clarified using 0.45 μm Millex-HV filters (Merck Millipore Ltd., Cork, Ireland). Samples for HPLC analyses were prepared by diluting 100 μL of the filtered extract with 400 μL of the mobile phase (pH 4.7 adjusted with 0.27 M citric acid) containing 50 mM NaH_2PO_4, 2.5 mM dodecyltrimethyl ammonium chloride and 1.25 mM disodium-dihydrogen-EDTA in water with 2% (v/v) addition of acetonitrile. An Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) was used for the HPLC analysis, equipped with an autosampler (4 °C), a quaternary pump, an in-line degasser, a column heater and a photodiode array detector. Separation was performed at 25 °C on a monolithic column; Chromolith® Performance RP-18e (100 mm × 4.6 mm i.d.) fitted with a Chromolith® RP-18e guard cartridge (5 mm × 4.6 mm i.d.) both obtained from Merck KGaA (Darmstadt, Germany). The flow rate was set to 0.05 mL min^{-1}. Detection of AsA was carried out at 264 nm. The results are expressed as mg of ascorbic acid per 100 g of fresh weight (FW) and calculated later on a dry weight (DW) basis.

For analyses of antioxidant capacity (AOC, determined by the Ferric Reducing Ability of Plasma (FRAP-assay)), total monomeric anthocyanins (TMA), and total phenolic compounds (TP), 30 g of berries were homogenized with a blender (Braun MR400, Karlsruhe, Germany) and 3 g of homogenate was extracted with 1 mM HCl (37%) in methanol (30 mL). The samples were flushed with nitrogen, capped, and vortexed (Vortex-T Genie 2, Scientific Industries Inc., Bohemia, NY, USA), followed by sonication at 0 °C for

15 min (Bandelin SONOREX RK 100, Bandelin Electronic GmbH & Co., Berlin, Germany). The liquid samples were stored at −20 °C until analysed. Prior to analysis, the samples were poured into a 2 mL microtube (Sarstedt, Nürnbrecht, Germany) and centrifuged at 13.200 rpm for 2 min at 4 °C (Eppendorf 5415 R, Hamburg, Germany). A KoneLab 30i (Thermo Electron Corp., Vantaa, Finland) was used for AOC, TMA and TP analysis. The AOC was determined by the FRAP-assay as described by Benzie and Strain (1996). TMA was determined by the pH-differential method based on the spectral characteristics of anthocyanins (Giusti and Wrolstad, 2005), and TP was determined using the Folin–Ciocalteu method (Singleton et al., 1999). No corrections were made for the contribution of ascorbic acid in our FC essay. Results were calculated as μmol Fe^{2+} per g of fresh weight (AOC), g of cyanidin-3-glucoside equivalents (CGE) per kg of fresh weight (TMA), g of gallic acid equivalents (GAE) per kg of fresh weight (TP), and were all later calculated on a dry weight (DW) basis.

2.3. Statistical analysis

The experiment was fully factorial, with a split-plot design, with temperature as main plots, and daylength and cultivar as sub-plots. The experiment was replicated with four randomised blocks, each consisting of two plants of the four cultivars in each treatment (i.e. 8 plants per treatment). One berry sample from each replicate, consisting of two plants, was analysed in each treatment. A three-factor fixed effect model was used to analyse the data. Effects of cultivar (fixed effect, four levels), temperature (fixed effect, three levels), and daylength conditions (fixed effect, three levels) were analysed by the General Linear Model (GLM) and Tukey's multiple comparison test with significance level $\alpha = 0.05$. To indicate the significant interactions between the investigated factors, data means are presented together with bars representing 95% confidence intervals (CI). Treatment results were considered as significantly different if the confidence interval of one did not overlap the others (Di Stefano, 2004). However, additional decomposition and one-way ANOVA tests within cultivar (differences between temperatures) were performed to investigate some of the cultivar × temperature interactions. Calculations were performed using a Minitab® 16 Statistical Software Package (Minitab Inc., State College, PA, USA).

3. Results

Berry ripening was delayed with increasing temperature for all cultivars (Table 1; Fig. 3C–E). Berries of 'Imandra' and 'Ben Tron' grown at 12 °C were harvested after 16 days of treatment, while 'Hedda' and 'Narve Viking' after 20 days, while at 18 °C and ambient conditions, berries of the same cultivars achieved full maturity after 18 to 22 days. At 24 °C, 'Imandra', 'Hedda' and 'Narve Viking' were harvested after 23 days, and the berries of 'Ben Tron' after 22 days (Table 1). At 24 °C not all berries became fully ripe, and only the most mature berries (75–80%) were sampled for further analysis (Fig. 3C–E). Berry yields of the different cultivars varied between 250 and 450 g per plant independent of post-flowering climate conditions. Berry size varied among the investigated cultivars, with 'Imandra' having the lowest average berry weight (1.35 g), followed by 'Narve Viking' (1.48 g), 'Ben Tron' (1.52 g) and 'Hedda' (1.90 g). These differences were significant at $p < 0.001$.

The genetic background of the investigated black currant cultivars had significant impacts on the chemical composition of their berries. The most prominent examples of genotypic influence was a 2.3 fold difference in AsA and a 2.1 fold for TP (Table 2), generally confirming earlier reports of cultivars ranks of fruit quality (Heiberg et al., 1992; Remberg et al., 2007; Krüger et al., 2011; Nes et al., 2012). Also, under controlled environment conditions, marked cultivar variations in all physicochemical fruit parameters were noted both under controlled and ambient conditions (Tables 2 and 3). 'Ben Tron' had the highest AOC and concentration of TP, in addition to the lowest TA. 'Hedda' was characterized by the lowest AsA, OD, AOC, TMA and TP. 'Imandra' had the highest TA, AsA and TMA and the lowest DM concentration, SS, and SS/TA ratio, while berries of 'Narve Viking' had the highest SS and DM concentration (Table 2). However, under ambient conditions, the lowest SS was observed in 'Imandra' (Table 3).

Temperature during berry ripening influenced all investigated physicochemical parameters of black currants, except for soluble solids (Table 2). Changes in fruit composition, however, showed only in a few cases, a strong and consistent response to the temperature gradient. TA increased with increasing temperature, thereby giving a reversed pattern for SS/TA ratio. The highest TA and increased DM content characterized berries grown at 24 °C (Table 2). Moreover, the warmest ripening conditions caused a decrease in OD and AOC as well as the concentrations of TMA. Berries ripened under field conditions had higher concentration of TMA and lower concentration of AsA than berries from any of the controlled temperature treatments, but did not vary significantly from these in their composition otherwise (Tables 2 and 3). Berries matured at 12 °C had significantly higher AsA concentration than berries from 18–24 °C.

Under identical daily light energy regimes, photoperiodic conditions (night interruption) had no significant effect on the chemical composition of black currant berries (Table 2). However, berries matured under natural day-length conditions had significantly lower pH and higher concentrations of TMA than berries from the other photoperiodic treatments, apparently, due to a 9% larger daily light integral of the former treatment. Also the concentration of SS and the SS/TA ratio tended to increase under natural LD conditions.

Significant cultivar × temperature (CV × TEMP) interactions were observed for all quality parameters, except for the SS/TA ratio and the AOC (Table 2). Details of some of the CV x TEMP interactions are presented in Fig. 4. 'Hedda' and 'Imandra' had the most stable DM concentration across the different ripening temperatures (Fig. 4A). The interactions for SS showed a similar pattern of decreasing concentrations with elevated temperature for 'Hedda' and 'Imandra', whereas for 'Ben Tron' the lowest SS was observed at 18 °C (Fig. 4B). Increasing acidity with increasing temperature was observed for all cultivars except for 'Hedda', which was most acidic at 18 °C (Fig. 4C). A general decrease in AsA concentration with increased temperature, described as the main effect, was not observed for the cultivar 'Imandra', while AsA appeared to be particularly vulnerable to high temperature in 'Narve Viking' (Fig. 4D). Accumulation of TMA was affected by temperature in a similar way (the highest concentration at 18 °C) in all cultivars investigated, although 'Hedda' had a more stable TMA than the other cultivars (Fig 4E). The effects of temperature on TP concentration of the various cultivars shown in Fig. 4F, indicates distinct temperature response patterns for each cultivar. A significant cultivar × daylength (CV × DAY) interaction was also observed for the concentration of TMA as well as a significant temperature × daylength (TEMP × DAY) interaction for the concentrations of DM, SS, TA and TMA (Table 2).

4. Discussion

The results are in general agreement with previous investigations, where large variations in important phytochemicals in different black currant cultivars and selections were observed (Nour et al., 2011; Krüger et al., 2011; Vagiri et al., 2013; Woznicki et al., 2015), supporting the claim, that genotype is the predominant factor determining fruit quality in black currants. The yield poten-

Table 2
Effects of temperature and daylength on the range of quality parameters determined in black currant berries. Data are the means (main effects) of four replications for cultivar, temperature and daylength treatments, respectively, and their degree of interaction.

		DM (%)	SS (%)	TA (%)	SS/TA ratio	pH	OD	AsA (mg/10 g DW)	AOC (mmol/10 g DW)	TMA (mg CGE/10 g DW)	TP (mg GAE/10 g DW)
Cultivar effect	'Ben Tron'	14.27 b	12.35 c	3.61 d	3.44 a	3.03 a	2.71 ab	98.23 b	5.08 a	86.36 b	367.0 a
	'Hedda'	13.97 b	13.06 b	3.86 c	3.38 ab	3.01 a	2.42 c	44.97 c	2.62 d	58.87 d	182.1 d
	'Imandra'	11.36 c	11.31 d	5.11 a	2.22 c	2.80 c	2.75 a	105.49 a	4.65 b	102.92 a	320.0 b
	'Narve Viking'	16.50 a	14.30 a	4.29 b	3.34 b	2.97 b	2.60 b	94.60 b	3.81 c	73.01 c	279.3 c
	Significance	***	***	***	***	***	***	***	***	***	***
Temperature effect	12 °C	13.89 b	12.92 a	3.98 c	3.31 a	2.96 a	2.67 a	90.19 a	4.14 a	81.80 b	289.4 ab
	18 °C	13.90 b	12.68 a	4.21 b	3.07 b	2.93 b	2.67 a	84.75 b	4.17 a	91.21 a	291.6 a
	24 °C	14.28 a	12.65 a	4.46 a	2.91 c	2.95 a	2.52 b	82.53 b	3.81 b	67.85 c	280.3 b
	Significance	**	n.s	***	***	**	***	***	***	***	*
Daylength effect	Natural LD	14.15 a	12.98 a	4.23 a	3.14 a	2.93 b	2.65 a	83.71 a	3.97 a	84.64 a	291.5 a
	SD	13.98 a	12.79 ab	4.22 a	3.10 ab	2.96 a	2.62 a	86.97 a	4.08 a	77.48 b	285.1 a
	SD + NI	13.94 a	12.49 b	4.20 a	3.05 b	2.97 a	2.58 a	86.78 a	4.07 a	78.84 b	284.7 a
	Significance	n.s	**	n.s.	**	***	n.s.	n.s.	n.s.	***	n.s.
Interactions	CVxTEMP	***	***	***	n.s	***	**	***	n.s.	**	**
	CVxDAY	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
	TEMPxDAY	**	*	*	n.s	n.s	n.s	n.s	n.s	**	n.s
	CVxTEMPxDAY	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ambient control, mean ± standard deviation		15.2 ± 2.0	14.0 ± 1.3	4.55 = ± 0.7	3.16 ± 0.6	2.97 ± 0.1	2.75 ± 0.1	78.7 ± 20.5	4.09 ± 0.9	100.2 ± 22	290.7 ± 67

Abbreviations used: DM—dry matter, SS—soluble solids, TA—titratable acidity, OD—optical density, AsA—ascorbic acid, AOC—antioxidant capacity, TMA—total monomeric anthocyanins, TP—total phenolics, SD—short day. Means that do not share a letter are significantly different at $p < 0.05$ level, with comparisons performed using Tukey's test. Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; n.s. = not significant.

Table 3
Fruit quality parameters for four black currant cultivars grown under ambient (outdoor) conditions. Data are the means of four replications, each consisting of two plants of each cultivar.

	DM (%)	SS (%)	TA (%)	SS/TA ratio	pH	OD	AsA (mg/10 g DW)	AOC (mmol/ 10 g DW)	TMA (mg CGE/10 g DW	TP (mg GAE/10 g DW)
'Ben Tron'	15.40 b	13.95 a	4.02 c	3.47 a	3.04 a	2.82 a	90.75 ab	4.95 a	108.5 ab	358.5 a
'Hedda'	15.50 b	14.98 a	4.24 bc	3.54 a	3.08 a	2.60 b	45.38 c	2.70 b	72.3 c	189.4 c
'Imandra'	12.27 c	12.01 b	5.69 a	2.13 b	2.77 b	2.78 a	97.56 a	4.67 a	126.5 a	320.8 ab
'Narve Viking'	17.63 a	14.90 a	4.25 b	3.51 a	3.00 a	2.75 a	80.97 c	4.03 a	93.5 bc	294.4 b
Significance	***	***	***	***	***	**	***	***	***	***

Abbreviations used: DM—dry matter, SS—soluble solids, TA—titratable acidity, OD—optical density, AsA—ascorbic acid, AOC—antioxidant capacity, TMA—total monomeric anthocyanins, TP—total phenolics. Means that do not share a letter are significantly different at $p < 0.05$ level, with comparisons performed using Tukey's test. Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

tial of black currant is mainly determined by the environmental conditions during floral initiation in the previous season (Sønsteby and Heide, 2011), and therefore, berry yield was not affected by post-flowering environment. However, interesting effects of post-flowering climatic environment was also observed.

In concurrence with our earlier findings under field conditions (Woznicki et al., 2015), berry concentrations of AsA decreased with increasing temperature in all cultivars tested (Table 2), although the effect was less marked in 'Imandra' than in the other cultivars (Fig. 4). The low AsA concentration found in berries grown under ambient conditions (Tables 2 and 3; Fig. 2), where the day temperature were sometimes well above 30 °C, also support the hypothesis of reduced AsA accumulation under elevated temperature conditions. Accumulation of L-AsA occurs in situ in black currant berries via the L-galactose pathway (Hancock et al., 2007), and is proposed to be regulated by post-transcriptional or post-translational mechanisms (Walker et al., 2010). Under natural growing conditions, accumulation of AsA occurs mainly during the early stages of berry development, and thereafter, decreases during the ripening (when presented on a fresh weight basis) (Rubinskienė et al., 2008). However, Viola et al. (2000) showed that, on a fresh weight basis, AsA concentration was relatively constant during growth and ripening, indicating, that only a dilution effect is responsible for lower AsA concentration in mature fresh fruits (cf. Remberg et al., 2010).

AsA is a potent antioxidant, with increased accumulation in plant tissues during oxidative stress, possibly due to overexpression of GDP-Mannose 3′, 5′-epimerase (L-Galactose pathway) as shown in tomato (Zhang et al., 2011). We may therefore, hypothetically assume, that lower temperatures (12 °C) during berry ripening might act as an oxidative stress factor, thereby causing the increased concentration of ascorbic acid. On the other hand, molecular research conducted on tomato showed that AsA concentration in fruits can be reduced by high temperature through inhibition of recycling of AsA from the oxidised form of ascorbate (Massot et al., 2013). Our previous results from field experiments (Woznicki et al., 2015), also indicated that black currant, being an understory shrub of low temperature origin, respond to elevated temperature with reduced fruit AsA concentration. Possibly, mechanisms of inhibited recycling may play a role in the regulation of AsA accumulation. A decrease in AsA concentration in fruits grown at high temperatures have been reported previously also in other crops (Lee and Kader, 2000; Richardson et al., 2004; Remberg et al., 2010), and may be of general occurence.

Accumulation of TMA was also reduced by high temperature with an optimum at 18 °C (Table 2). Delayed ripening and colour change at 24 °C also indicated suppression of anthocyanin biosynthesis at high temperatures. It is known, that low temperature during ripening is an inducing factor for the expression of key genes controlling anthocyanin biosynthesis, such as CHS (chalcone synthase), ANS (anthocyanidin synthase) and UFGluT (UDP-glucose: flavonoid 3-O-glucosyltransferase) (Ubi et al., 2006). Mori et al. (2005) observed that grapes grown under higher temperatures accumulated less anthocyanins, possibly due to a negative influence of elevated temperature on the stability of intermediates (e.g. anthocyanidin) as well as enzymes activity.

Control plants grown under ambient conditions were characterised by high concentrations of TMA (Table 2). Therefore, considering the relatively high ambient temperature during the experiment (Fig. 2), the elevated TMA concentration might be due to a positive influence of lower night temperature on transcription factors regulating anthocyanin accumulation in fruits. The sensitivity of anthocyanin biosynthesis to temperature was demonstrated in a field experiment with apples, where only a single night of lower temperature was sufficient to increase transcription of the MYB10 factor, and in consequence, enhancement of anthocyanin biosynthesis (Lin-Wang et al., 2011).

Reduced anthocyanin accumulation in fruit tissues exposed to elevated temperature during ripening, seems to be a rather general response (Mori et al., 2007; Jaakola and Hohtola, 2010; Lin-Wang et al., 2011), although some contradictory results were also reported (Wang and Zheng, 2001; Guerrero-Chavez et al., 2015; Vagiri et al., 2013). These results contradict with our results with black currant, and the main reports in the literature for a range of species, suggesting species specific differences, and in the case of black currant, possible interference by unknown factors on secondary metabolite accumulation.

TP and TMA have been reported as the main contributors to antioxidant capacity of many berries (Moyer et al., 2002; Remberg et al., 2010; Krüger et al., 2011), and this was supported by our results (Table 2). Most of the antioxidants in black currant fruits are found in the skin, and small berries have a higher skin to volume ratio, resulting in higher AOC (as well as TP and TMA). Thus, Krüger et al. (2011) reported a negative correlation between berry size and AOC. This is partly in accordance with our results, where 'Hedda' with the largest berries (1.90 g/berry), had the lowest AOC, TMA and TP. However, 'Imandra' which had the smallest berries (1.35 g), had the highest concentration of TMA but not TP and AOC.

Biosynthesis of anthocyanins in fruit crops is generally enhanced by increased PAR (Kliewer, 1970; Erez and Flore, 1986; Woznicki et al., 2015), and this is in agreement with the present results.

The enhanced SS concentration under natural daylength conditions may thus be a result of higher photosynthesis rates, and in consequence, higher sugar (SS components) production (Beckles, 2012). On the other hand, acidity increased with increasing temperature (Table 2), in agreement with the finding of Kaldmäe et al. (2013), that acidity of black currant berries increased in years with high average July temperature.

Under regimes of identical daily light energy, photoperiod had no significant effect on the chemical composition of black currant berries (Table 2). On the other hand, the additional 9% daily light energy received by the plants under natural LD conditions, resulted in significantly higher concentrations of TMA as well as significant cultivar × daylength and temperature x daylength interactions on this parameter (Table 2). This illustrates the importance of keeping the daily light energy supply constant in photoperiodic experiments of this kind in order to avoid confounding effects of

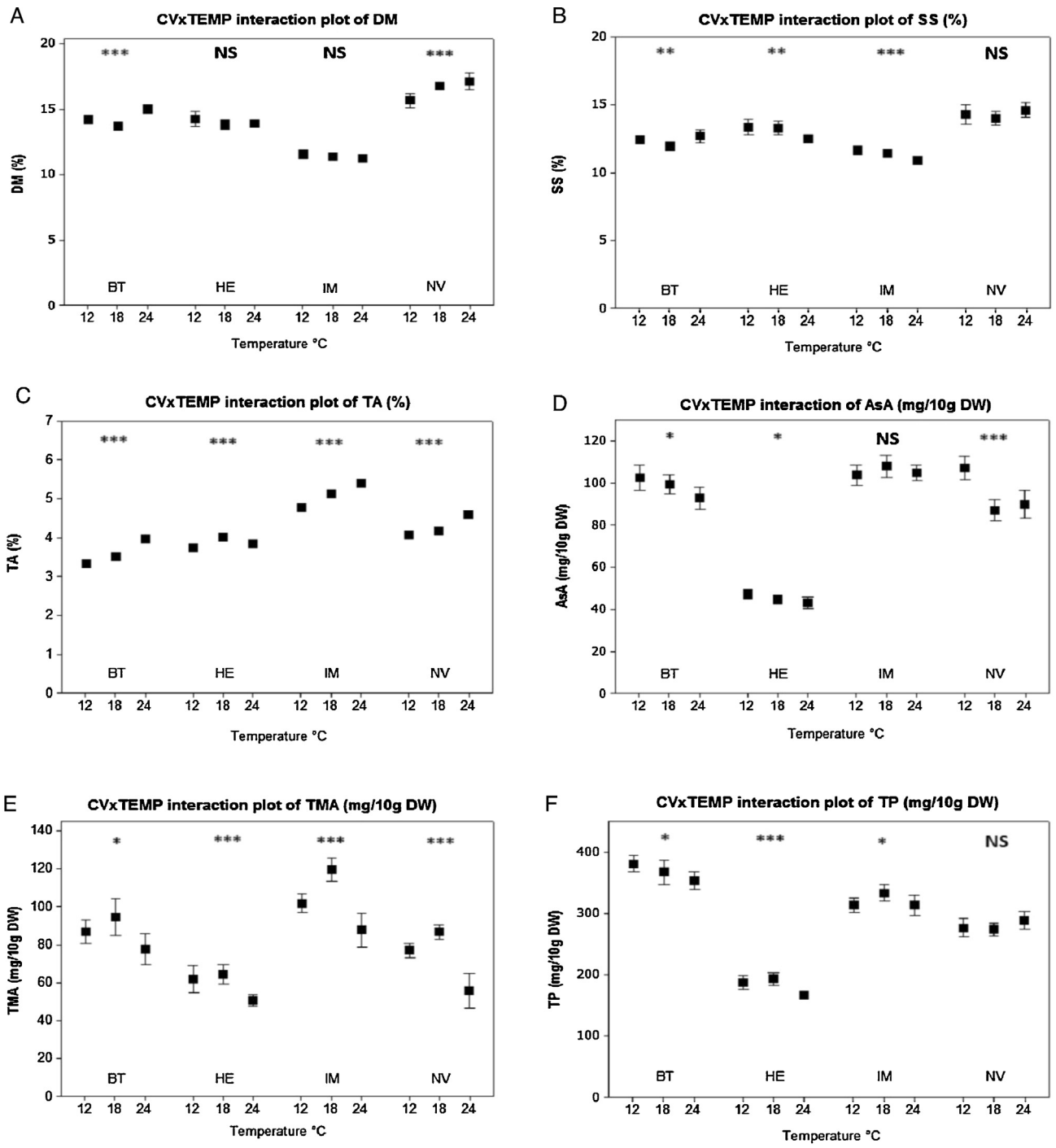

Fig. 4. Cultivar × temperature interaction plots for a range of fruit quality parameters as determined in black currant berries. Data are the means of all daylength treatments, and the vertical bars are representing 95% confidence intervals for the means. Abbreviations used: BT = 'Ben Tron', HE = 'Hedda', IM = 'Imandra', NV = 'Narve Viking', (A) DM—dry matter, (B) SS—soluble solids, (C) TA—titratable acidity, (D) AsA—ascorbic acid, (E) TMA—total monomeric anthocyanins, (F) TP—total phenolics. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant.

simultaneous changes in photoperiod and light energy supply (cf. Thomas and Vince-Prue, 1997).

The concentrations of TP and TMA observed in berries maturing under phytotron conditions were generally lower than in berries maturing under ambient conditions (Table 2), and lower than previously reported for black currants produced under field conditions (Skrede et al., 2012). Information about biosynthesis and accumulation of phenolic compounds in black currants is limited, while in other fruits, such as grapes and apples (Ubi et al., 2006; Fernandes de Oliveira et al., 2015), UV-radiation is known as an inducing factor of anthocyanin biosynthesis. Therefore, a reduction of 30–50% of UV radiation as measured under the glass barrier in the phytotron, might in part be responsible for the observed low concentrations of phenolic compounds. It is also possible that the constant environmental conditions in the phytotron, with lack of cool nights and extreme heat and drought stress, also might have resulted in less robust berries with thinner skin, and thereby, reduced TMA and TP concentrations (cf. Moyer et al., 2002; Krüger et al., 2011). These are factors that should be studied in further investigations.

Despite the limitations discussed above, the controlled environment approach, by which the effects of each climatic factor can be assessed separately and in combination with controlled gradients of other factors, seems the most promising one for disentangling the complex interacting effects of the range of climatic factors influencing fruit chemical composition.

In conclusion, the presented results confirm the previously reported findings (Walker et al., 2010; Nour et al., 2011; Krüger et al., 2011; Vagiri et al., 2013; Woznicki et al., 2015), that plant genetic constitution commonly is of greater importance than the climatic environment for the chemical composition of black currant fruits. However, the results also demonstrate that the environmental factors prevailing during fruit maturation, in particular temperature, can significantly change fruit chemical composition regardless of the genetic constitution of the cultivars. Important quality components such as AsA, AOC and TMA, were all reduced by increasing temperature, whereas TA increased. Under constant energy regimes, photoperiod had no specific effect on any of the fruit quality components studied, while a 9% increase in daily light integral received by plants grown under natural long day conditions significantly enhanced TMA accumulation. The study provides evidence that black currant fruit accumulation of AsA and TMA is favoured by low post-flowering temperatures, and that high daily light integrals also tend to favour TMA biosynthesis.

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Paper III

TITLE

Ascorbate pool, sugars and organic acids in black currant *(Ribes nigrum* L.) berries are strongly influenced by genotype and post-flowering temperature

RUNNING TITLE

Temperature effects on ascorbate pool, sugars and acids in black currant

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ABSTRACT

BACKGROUND: Marked effects of the climatic environment on fruit chemical composition has often been demonstrated in field experiments. However, complex covariations of several climatic factors in the natural environment complicates the interpretation of such experiments and the identification of the causal factors. This can better be done in a phytotron where the various climatic factors can be varied systematically. Therefore, we grew four black currant cultivars of contrasting origin in a phytotron under controlled post-flowering temperature and photoperiod conditions and analysed the berries for their contents of ascorbic acids, sugars and organic acids.

RESULTS: The analyses revealed significant effects of genotype on all investigated compounds. Particularly large cultivar differences were observed in the concentrations of L-ascorbic acid (AA) and sucrose. The concentrations of both AA and dehydroascorbic acid (DHAA), as well as the concentrations of all major sugars decreased consistently with increasing temperature over the 12 - 24 °C range. Fructose and glucose were the predominant sugars with concentrations several fold higher than those for sucrose. AA was the main contributor to the total ascorbate pool in black currant berries, the AA/DHAA ratio varied from 5.6 to 10.3 among the studied cultivars. The concentration of citric acid, which was the predominant organic acid in black currant berries, increased with increasing temperature, while the opposite trend was observed for malic and shikimic acid. Quninic acid was always present at relatively low concentrations. On the other hand, photoperiod had no significant effect on berry content of any of the investigated compounds.

CONCLUSSION: It is concluded that post-flowering temperature has marked effects on the concentration of important chemical compounds responsible for taste and nutritional value of black currant berries, whereas photoperiod has no such effect in the studied cultivars.

KEYWORDS:

dehydroascorbic acid, L-ascorbic acid, organic acids, photoperiod, sugars, temperature

INTRODUCTION

Black currant (*Ribes nigrum* L.) is an important soft fruit crop for the cold and temperate regions and is known for its high nutritional and health promoting properties.[1] The berries are an excellent source of ascorbic acid (vitamin C) as well as various phenolic compounds, free sugars and organic acids.[2,3]

Free sugars and organic acids in fruits are important components of taste, which together with aroma determine fruit sensorial value.[4] Complex interplays between sugar and acid metabolisms govern development and stabilization of final fruit quality. Immature berries accumulate organic acids, using sugars imported from the leaves as a metabolic energy source. During ripening, berries turn into a 'sink' for sugars. Additionally, main soluble sugars are the precursors of ascorbic acid biosynthesis in black currant.[5]

Ascorbic acid is an essential component in the human diet, and cannot be synthesized within the body. The concentration of vitamin C in commercial black currant cultivars can be as high as 300 mg/100 ml of juice, while, breeding lines with concentrations up to 350 mg/100 mg fresh weight are also known.[6,7] As a strong antioxidant, vitamin C can mitigate the harmful effects of oxidative stress on cells and tissues.[8] In acting as an antiscorbutic factor, vitamin C plays a crucial

role in the biosynthesis of collagen, production of cholesterol, some peptide hormones and L-carnitine.[9,10] Additionally, vitamin C may have an important role in reducing the risk of cancer[11] and cardiovascular diseases.[12,13]

The content of L-ascorbic acid (AA) in black currants is influenced by both genetic and environmental factors.[14-16] Environmental conditions such as temperature, solar radiation and water availability may play an important role in accumulation of ascorbic acid, both its reduced and oxidized forms and their precursors.[17] In black currant, AA is produced via the L-galactose pathway and takes place *in situ* in the berry tissue.[5,18] Ascorbate concentration in plant tissues depends on the rate of biosynthesis, oxidation and recycling.[19] It is known, that ascorbate peroxidase (APX) catalyses the oxidation of AA into monodehydroascorbic acid (MDHA), a short-lived radical rapidly converted into AA and dehydroascorbic acid (DHAA). The enzymatic regeneration of AA from MDHA and DHAA is catalysed by monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), the latter being the key reaction in the so-called ascorbate–glutathione cycle, using reduced glutathione (GSH) as a source of reductant.[20] Recycling of AA leads to neutralisation of H_2O_2.[21] The ratio of AA and DHAA depends on the redox state of the plant cells,[22] and can be influenced by various abiotic conditions, leading to changes in the expression of DHAR.[23]

The structures of AA and DHAA require different ways of intestinal absorption in mammals. AA is transported by the sodium-dependent vitamin C transporter 2 (SVCT2), while DHAA by the facilitated-diffusion glucose transporters, GLUT 2 and GLUT 8,[24] which ensure uptake of vitamin C into cells that do not have vitamin C transporters. After oral application of DHAA, plasma concentrations of ascorbate peaks faster than if applied as AA, because DHAA is rapidly

reduced to AA.[25] This implies the importance of investigating both AA and DHAA concentrations when studying food plants, as DHAA represent a significant part of the total ascorbate pool.

In an eight-year field study in Norway, we found that berry concentration of AA in black currant was negatively correlated with summer temperature while precipitation had the opposite effect.[16] In a recent paper,[26] we further reported that berry concentration of AA decreased with increasing temperature over the 12 – 24 °C range also under controlled environment conditions, whereas photoperiod had no significant effect. On the contrary, a positive relationship between post-flowering temperature and AA concentration was observed during a long term field experiment conducted in UK.[14] It was also reported that black currants grown in southern (warmer) locations in Sweden had higher AA concentrations than those grown at northern locations.[7] However, it is difficult to interpret the specific effect of the various climatic factors under field conditions where simultaneous changes in several factors usually take place, resulting in complex covariations. The untangling of this complexity will usually require deeper analyses also under controlled environment conditions.[26-28]

The aim of the present study was thus to investigate the effect of controlled post-flowering temperature and photoperiod on the total ascorbate pool, comprising AA and DHAA. Further, to examine treatment effects on soluble sugars (ascorbate precursors in the L-galactose biosynthesis pathway), and major organic acids which are important contributors to the sensory properties of the berries.

MATERIALS AND METHODS

Chemicals

Acetonitrile, L-(+)-ascorbic acid (AA), citric acid, sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{}^*2H_2O$), disodium hydrogen phosphate dihydrate, disodium EDTA and n-dodecyltrimethylammonium chloride were obtained from Merck KGAa (Darmstadt, Germany). Dehydro-L-(+)- ascorbic acid dimer (DHAA), malic acid, quinic acid, shikimic acid and tris[2-carboxyethyl]-phosphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Metaphosphoric acid was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Glucose, fructose and sucrose were purchased from Chem Service (West Chester, PA, USA). All solvents were of HPLC grade and water was of Milli-Q-quality (Millipore Corp., Bedford, MA, USA).

Plant material and cultivation

Origin of the four cultivars used, and their cultivation during plant raising and experimentation, as well as the physical conditions in the daylight phytotron where the experiment was conducted, are all explained in detail in our earlier paper.[26] The cultivars were the high-boreal Russian cultivar Imandra (IM), originating from the Kola Peninsula (67º30'N), Hedda (HE) and Narve Viking (NV) from the Norwegian breeding program at Ås (59º40'N) and Ben Tron (BT) from the Scottish breeding program in Dundee (56º30'N). During the last three weeks of berry maturation, the plants were exposed to constant temperatures of 12, 18 and 24 °C (± 1 °C) combined with the following photoperiodic conditions: 1) Natural long summer day light, ca. 19 h (Natural LD), 2) 10 h summer daylight (SD), and 3) 10 h SD + 3 h night interruption (SD + NI). Both treatment 1) and 3) are perceived as LD conditions by the plants, but the former provided a 9% larger daily light integral. On the other hand, by using low intensity incandescent lamps for the night interruption (approx. 7

μmol quanta m^{-2} s^{-1}), the daily light integral varied by less than 0.5% between treatment 2) and 3) which represent the true photoperiodic test. As a control, plants of all cultivars were also grown outdoors under ambient summer light and temperature conditions (59°40'N). During the experimental period the outdoor daily mean temperature varied between 18 and 25 °C. No precipitation was recorded during this period. Berries were hand harvested when fully ripe, based on berry softness and visual assessment of colour. They were immediately frozen and stored at -20 °C until analysed.

Experimental design and statistical analysis

The experiment was fully factorial with a split-plot design, with temperatures as main plots and photoperiod and cultivar as sub-plots. The experiment was replicates with four randomized blocks, each comprising two plants of each cultivar on a separate trolley, giving eight plants per treatment. One berry sample (200 – 500 g) of each cultivar from each plant treatment replicate was analysed. A three-factor fixed effect model was used to analyse the data. Effects of cultivar (CV, fixed effect, four levels), temperature (TEMP, fixed effect, three levels), and daylength conditions (DAY, fixed effect, three levels) were analysed by General Linear Model (GLM) and Tukey's multiple comparison test with significance levels $\alpha = 0.05$. Where significant interactions between the investigated factors was present, data means are presented together with bars representing 95% confidence intervals (CI). Treatment results were considered as significantly different if the confidence interval of one did not overlap the others.[29] However, additional decomposition and one-way ANOVA tests within cultivar (differences between temperatures) were performed. Pearson's correlation coefficients were used to test the relations between ascorbic acid and sugars. All calculations were performed using a Minitab® 16 Statistical Software Package (Minitab Inc.

State College, PA, USA). A few outliers with values > 50% off the means, were removed from the calculations.

Determination of ascorbic acids

AA and DHAA concentration were determined according to Aaby et al.[30] with some modifications. Briefly, ascorbic acids in homogenized, frozen berries (5 g) were extracted with 5% meta-phosphoric acid containing 1 mM disodium-dihydrogen-EDTA (50 mL). Samples for AA determination (100 µL) were diluted with mobile phase (400 µL) prior to analysis. To determine total ascorbic acid, DHAA in the extracts (100 µL) was reduced with 5 mM TCEP, pH 9.0 (50 µL) and diluted with mobile phase, pH 4.2 (350 µL) prior to analysis. Analysis of AA in the samples (15 µL) was performed on an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a thermo-stated autosampler (4 ^{o}C), a quaternary pump, an in-line degasser, a column heater and a photodiode array detector (DAD). Separation was performed at 25 °C on a monolithic column; Chromolith® Performance RP-18e (100 mm × 4.6 mm i.d., Merck KGaA, Darmstadt, Germany) fitted with a Chromolith® RP-18e guard cartridge (5 mm × 4.6 mm i.d., Merck KGaA) with mobile phase 2.5 mM $Na_2H_2PO_4$*2 H_2O, 2.5 mM *n*-dodecyltrimethylammonium chloride, 1.0 mM Na2-EDTA, and 2% acetonitrile, adjusted to pH 4.7 with 0.27 M citric acid. The flow rate was 1 mL min^{-1}. AA was detected at 264 nm and quantified by use of external standard. Total ascorbic acids in the samples were determined after reduction of DHAA to AA. The concentration of DHAA was calculated by subtracting the AA concentration from the total ascorbic acid concentration. The results were expressed as g AA, DHAA or total ascorbic acid per kg dry weight (DW).

Analysis of organic acids and sugars

Thawing berries were homogenized in a food processor (CombiMax 700, Braun GmbH, Kronberg, Germany). After centrifugation (18000 rpm, 10 min, 5 °C; Avanti J-26XP centrifuge, Beckman Coulter, Fullerton, CA, USA) the supernatant (1 mL) was diluted with water (3 mL) and filtered (0.45 μm Millex-HV filters, Merck Millipore Ltd., Cork, Ireland) prior to HPLC analysis. Samples (20 μL) were injected on an Agilent 1100 Series HPLC (Agilent Technologies) equipped with an autosampler cooled to 4 °C, a DAD and a refractometer index (RI) detector (Model 132, Gilson, Villiers-le-Bel, France). Separation was performed on a Rezex ROA-Organic acid H^+ (8%) column (300 mm x 7.8 mm, Phenomenex, Torrance, CA, USA) at 45 °C with mobile phase 7.2 mM H_2SO_4 and flow rate 0.5 mL/min. Glucose, sucrose and fructose were detected with RI detection and the organic acids with DAD at 210 nm. Identification of the compounds was based on comparison and spiking with authentic standards. Quantification was done by use of external standard curves. The results were expressed as g kg^{-1} dry weight (DW).

RESULTS

There were highly significant differences among the cultivars in the concentration of all the analysed compounds (Tables 1, 2 and 3). Most notable were the low concentrations of individual and total ascorbic acids in Hedda and the low concentration of sucrose in Imandra which also had the highest concentration of most acids, including the ascorbic acids. AA was the main contributor to the total ascorbate pool in all cultivars, the AA/DHAA ratio varying from 5.6 (Hedda) to 10.3 (Narve Viking) among the four cultivars. Total ascorbic acid and its two forms, as well as the ratio of AA to DHAA, all decreased markedly with increasing temperature over the 12 – 24 °C range (Table 1). In berries matured under ambient outdoor conditions, the concentrations of AA and total ascorbic acid were comparable with those observed at 24 °C in the phytotron (Table 1). Under

identical light energy conditions, daylength had little or no effect on AA and DHAA, whereas their total concentration was lower under natural LD conditions than in either day-length with lower daily light integrals. There was, however, a highly significant interaction between cultivar and temperature on AA as well as total ascorbic acid concentrations (Table 1, cf. Fig. 1).

Citric acid, followed by malic acid, were the predominant organic acids in all cultivars. Both were present in higher concentrations in Imandra than in the other cultivars (Table 2). While citric acid concentration increased with increasing temperature, malic acid showed the opposite trend, its concentration being halved over the 12 – 24 °C temperature range. Hence, the citric/malic acid ratio increased several-fold with increasing temperature. Quinic acid and shikimic acid were present in low concentrations, and while the former was little influenced by temperature, shikimic acid concentration decreased by 25 % with increasing temperature over the total temperature range. As with ascorbate concentrations, the concentrations of all the organic acids in berries matured under natural outdoor conditions were similar to those recorded at 24 °C in the phytotron. Photoperiod had no significant effect on the organic acid concentrations, while there was a highly significant temperature x daylength interaction effect on the concentrations of malic and quinic acid. (Table 2, cf. Fig. 2).

In addition, the sugar concentrations varied considerably among the cultivars. The greatest differences were noted for sucrose which was extremely low in Imandra, (undetectable at high temperature), whereas Hedda and Narve Viking were relatively rich in sucrose (Table 3). The concentrations of the monosaccharides fructose and glucose also varied among the cultivars, but to a lesser extent than did the sucrose. The low sucrose concentration in Imandra was associated with the highest concentration of fructose, thus rendering the sum of sugars less variable than the individual sugars. On average for all cultivars, all the sugars decreased significantly with

increasing temperature giving a reduction of 27 % in total sugars over the entire temperature range. Since the negative effect of temperature on sucrose concentration was much larger in Hedda and lower in Imandra than in the other cultivars, there was also a significant cultivar x temperature interaction effect on the sucrose concentrations (Table 3, Fig. 3). Under outdoor conditions, both individual and total sugar concentrations resembled those at 18 °C in the phytotron. Photoperiod had no significant effect on the sugar concentrations of these black currant cultivars (Table 3).

DISCUSSION

The present results confirm the results of earlier investigations showing that berry composition of black currants is strongly influenced by plant genotype, and to a lesser extent, by environmental conditions.[7,14-16] Particularly noticeable were the large cultivar differences in ascorbic acid and sucrose content, and the marked modifying effect of temperature on the contents of ascorbic acid as well as major sugars and organic acids (Tables 1, 2 and 3).

Although the sucrose content was generally much lower than those of glucose and fructose in all cultivars, the lowering effect of high temperature was almost the same. Since sugars are translocated within the plant in the form of sucrose, the low concentrations of sucrose in most berry fruits is apparently due to enzymatic hydrolysis after translocation from the leaves.[4] Our result show that lower growth temperature lead to increased accumulation of glucose, fructose and sucrose in black currant berries. This is in agreement with the results of Zheng et al.[31,32] with black currants and other *Ribes* spp. in Finland, as well as results with various other berries.[33,34] Wang et al.[35] suggested that sucrose synthase activity decreased with exposure to heat stress. However our results show that sucrose in berries grown under natural warm outdoor conditions was higher than in any of the controlled environmental conditions, while the concentrations of glucose and fructose

was similar for the outdoor and 18 ºC treated plants (Table 3) and the range of concentrations found were comparable with the results reported in other papers.[31,32]

Black currants are characterized by relatively high concentration of ascorbic acid as compared to other berry crops.[7,36] Under natural growth conditions, accumulation of ascorbic acid started in the early stages of berry growth (from 4 to 12 mm diameter), with a plateau (influenced by the environment) during fruit maturation.[37] However, such a continuous decrease in ascorbic acid concentration during berry maturation was only observed when determined on a fresh weight basis,[38] since berry growth and ripening occur simultaneously and may lead to dilution of important phytochemicals. To avoid such biases, it is important to express the analysis data on a dry weight basis as done here.[27]

Biosynthesis and accumulation of AA are reported to be regulated by post transcriptional or post translational mechanisms.[14] Therefore, genetic background is an important factor modifying the plant responses to environmental conditions. Our results showed that ascorbic acid concentrations in the cultivars Imandra and Hedda were less influenced by post-flowering temperature than in the other cultivars used in this experiment (Fig. 1).

It is known that AA oxidation and recycling may be influenced by factors such as genotype, concentration, temperature, light and pH.[17,39,40] Therefore, it is not surprising, that also the ratio between the reduced and oxidized forms of ascorbic acid (AA/DHAA) was influenced by the experimental treatments. The results in Table 1 show a marked increase in the AA/DHAA ratio across the 12 – 24 °C temperature range, indicating a parallel increase in the oxidative state of the fruit cells. Being a potent antioxidant, AA tends to increase under oxidative stress, possibly due to overexpression of GDP-Mannose 3',5'-epimerase in the L-Galactose pathway of ascorbic acid biosynthesis.[5,41] Thus, Ioannidi et al.[19] found an accumulation of transcript L-Gal-1-phosphate

phosphatase (GPP) under cold stress (4 ^{o}C for 48 h) in tomato fruit. Moreover, Stevens et al.[42] also observed an increased accumulation of DHAA and a lower MDHA reductase activity in tomato fruit tissue under chilling stress. A similar increase in DHAA concentration at low temperature was also observed in black currant (Table 1). We may therefore assume that cool temperature during berry ripening (12 ^{o}C) in a similar way might have triggered the increased accumulation of ascorbic acids observed. When AA in fruit cells is oxidized as a result of its antioxidant function, it is usually rapidly recycled from DHAA.[20] However, high temperature can cause an inhibition of ascorbate recycling in fruits,[43] and in consequence, reduce AA accumulation. Finally, DHAA, if not recycled to AA, can be degraded to produce oxalate and threonate residues.[44,45] These processes may be the mechanisms underlying the observed reduction of the ascorbate pool and the associated increase of the AA/DHAA ratio under increasing post-flowering temperatures in black currant fruits (Table 1).

AA levels were significantly correlated ($p < 0.05$) with hexose sugars in the cultivars Ben Tron (Glucose: r = 0.429; Fructose: r = 0.513), Hedda (Glucose: r = 0.466; Fructose: r = 0.556) and Narve Viking (Glucose: r = 0.539; Fructose: r = 0.558) but not in Imandra. Zheng et al.[32] reported a positive correlation between AA and sucrose in black currant. Similarly, Tiitinen et al.[46] reported a positive correlation between glucose and AA concentration in sea buckthorn. Such relations suggest an important role of free sugars in the berries as precursors for ascorbic acid biosynthesis. The *in situ* location of the synthesis strengthens the probability of a causal connection underlying the relations.[5]

The accumulation of citric acid is responsible for the majority of black currant acidity.[38] In another study, decreased acidity as fruits ripened from green to black colour was shown.[47] The influence of temperature on fruit acidity was also shown by an experiment conducted in Estonia,

where acidity of black currant berries increased in years with higher average July temperatures.[48] Our results concur with these observations. Moreover, a similar response pattern was also described by Remberg et al.[27] for Glen Ample raspberries grown under controlled environment conditions.

Synthesis of malic acid involves an exothermic reaction,[49] and is therefore, favoured by lower temperatures in comparison to the degradation of the acid. This may explain the negative relation between the concentration of this organic acid and elevated temperature found in the present study and also previous reported for various fruit species.[50,51]

Correlation analysis performed by Zheng et al.[32] on black currants in Finland revealed a strong negative linear relation between quinic acid concentration and solar radiation pre harvest. However, in the present experiment, the quinic acid concentration was mainly influenced by plant genetic background, showing varying responses to temperature in different cultivars.

In conclusion, our present and earlier results[16,26] provide strong evidence that elevated post-flowering temperature causes a marked decrease in the concentration of ascorbic acid (both reduced and oxidized forms), sugars (glucose, fructose and sucrose), and simultaneously, increases citric acid and lowers malic acid concentrations in black currant fruits. We therefore conclude that cool summer conditions during ripening of black currant seem to favour the production of berries with high nutritional value and good taste. The results also revealed distinct cultivar differences in temperature stability for the accumulation of valuable berry constituents that may be of importance for future breeding of black currants with better taste and health-promoting properties.

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TABLES

Table 1. Effects of cultivar, temperature and daylength on L-ascorbic acid, dehydroascorbic acid, total ascorbic acid (g kg $^{-1}$ DW) and the ratio between L-ascorbic acid and dehydroascorbic acid in black currant berries.[a]

	Variables	L-AA[b]	DHAA	Total AA	L-AA/DHAA (ratio)
Cultivar effect	Ben Tron	9.9 b	1.7 ab	11.5 b	8.6 ab
	Hedda	4.4 d	0.9 c	5.3 d	5.6 b
	Imandra	10.5 a	1.8 a	12.3 a	6.6 b
	Narve Viking	9.4 c	1.3 b	10.7 c	10.3 a
	Significance[c]	***	***	***	**
Temperature effect	12 °C	8.9 a	1.9 a	10.8 a	5.2 c
	18 °C	8.5 b	1.4 b	9.8 b	7.7 b
	24 °C	8.2 b	1.1 c	9.3 c	10.3 a
	Significance	***	***	***	***
Daylength effect	Natural LD	8.3 a	1.4 ab	9.7 b	7.3 a
	SD	8.6 a	1.6 a	10.3 a	7.3 a
	SD + NI	8.7 a	1.3 b	10.0 ab	8.7 a
	Significance	NS	*	**	NS
Interactions	CV x TEMP	***	*	***	NS
	CV x DAY	NS	NS	NS	NS
	TEMP x DAY	NS	NS	NS	NS
	CV x TEMP x DAY	NS	NS	NS	NS
Ambient control: Mean ± standard deviation		7.9 ± 2.1	1.4 ± 0.7	9.3 ± 2.3	6.9 ± 3.4

[a]All data are means based on four replicates. [b]Means that do not share a letter are significantly different at $p < 0.05$ level, with comparisons performed using Tukey's test. [c]Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see the Materials and Methods section.)

Table 2. Effects of cultivar, temperature and daylength on the concentrations of citric, malic, quinic and shikimic acids (g kg $^{-1}$ DW) in black currant berries.[a]

	Variables	Citric acid[b]	Malic acid	Quinic acid	Shikimic acid
Cultivar effect	Ben Tron	137 c	31 c	15 ab	0.7 c
	Hedda	153 b	50 b	5 c	1.8 a
	Imandra	203 a	78 a	16 a	1.6 b
	Narve Viking	128 c	35 c	13 b	1.5 b
	Significance[c]	***	***	***	***
Temperature effect	12 °C	149 b	66 a	12 b	1.6 a
	18 °C	156 ab	48 b	13 a	1.4 b
	24 °C	161 a	31 c	12 b	1.2 c
	Significance	**	***	*	***
Daylength effect	Natural LD	151 a	47 a	12 a	1.3 a
	SD	158 a	48 a	13 a	1.4 a
	SD + NI	158 a	49 a	12 a	1.4 a
	Significance	NS	NS	NS	NS
Interactions	CV x TEMP	NS	***	***	NS
	CV x DAY	NS	NS	NS	NS
	TEMP x DAY	NS	NS	NS	NS
	CV x TEMP x DAY	NS	NS	NS	NS
Ambient control: Mean ± standard deviation		155 ± 30	43 ± 26	14 ± 4	1.3 ± 0.4

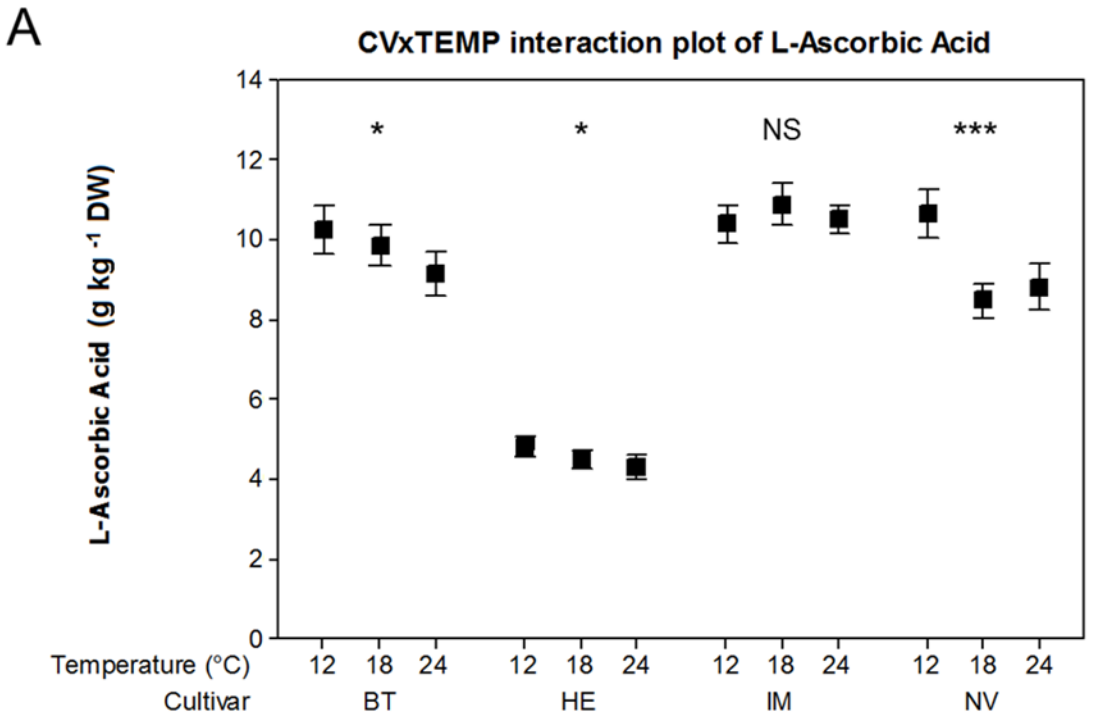
[a]All data are means based on four replicates. [b]Means that do not share a letter are significantly different at $p < 0.05$ level, with comparisons performed using Tukey's test. [c]Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see the Materials and Methods section.)

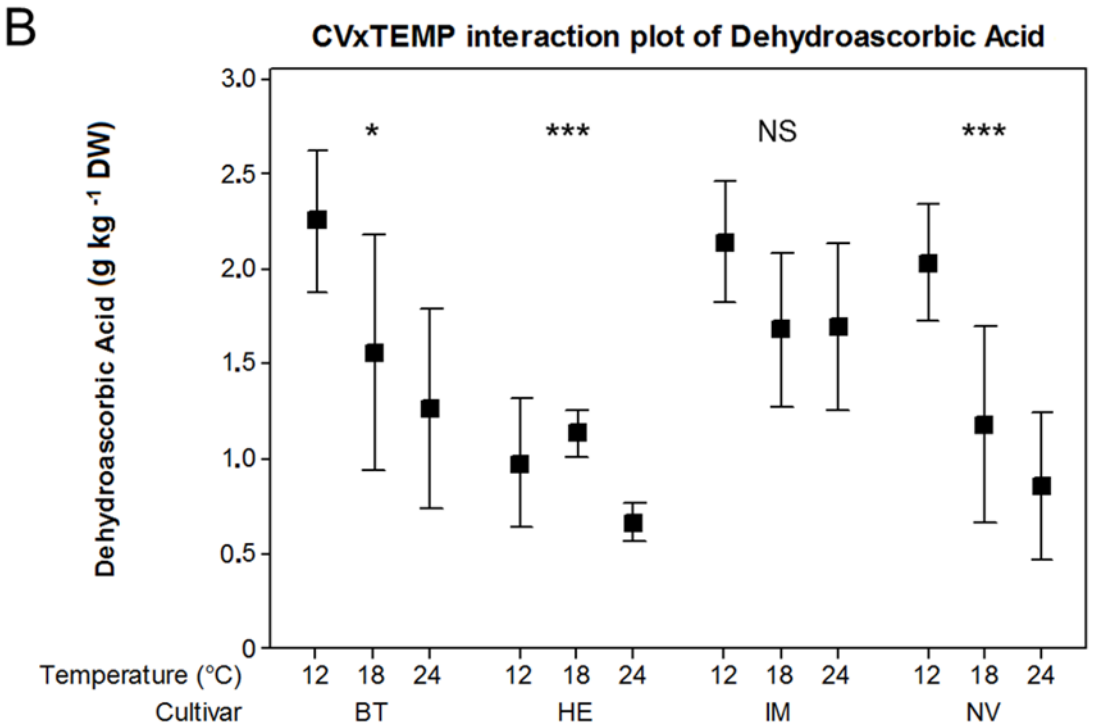
Table 3. Effects of cultivar, temperature, and daylength on the concentrations of glucose, fructose, sucrose and total sugars (g kg $^{-1}$ DW) in black currant berries.[a]

	Variables	Glucose[b]	Fructose	Sucrose	Total sugars
Cultivar effect	Ben Tron	166 b	256 b	18 c	440 b
	Hedda	203 a	243 b	42 b	487 a
	Imandra	147 c	285 a	6 d	437 b
	Narve Viking	167 b	243 b	58 a	467 ab
	Significance[c]	***	***	***	**
Temperature effect	12 °C	194 a	289 a	37 a	520 a
	18 °C	167 b	255 b	29 b	451 b
	24 °C	150 c	226 c	26 b	402 c
	Significance	***	***	**	***
Daylength effect	Natural LD	177 a	263 a	32 a	473 a
	SD	168 a	255 a	31 a	455 a
	SD+NI	166 a	252 a	29 a	447 a
	Significance	NS	NS	NS	NS
Interactions	CV x TEMP	NS	NS	***	NS
	CV x DAY	NS	NS	NS	NS
	TEMP x DAY	NS	NS	NS	NS
	CV x TEMP x DAY	NS	NS	NS	NS
Ambient control: Mean ± standard deviation		171 ± 31	256 ± 28	40 ± 28	467 ± 40

[a]All data are means based on four replicates. [b]Means that do not share a letter are significantly different at $p < 0.05$ level, with comparisons performed using Tukey's test. [c]Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see the Materials and Methods section.)

FIGURES


A
CVxTEMP interaction plot of L-Ascorbic Acid
L-Ascorbic Acid (g kg -1 DW)
14
12
10
8
6
4
2
0
*
*
NS
***
Temperature (°C)
12 18 24 12 18 24 12 18 24 12 18 24
Cultivar
BT HE IM NV


B
CVxTEMP interaction plot of Dehydroascorbic Acid
Dehydroascorbic Acid (g kg -1 DW)
3.0
2.5
2.0
1.5
1.0
0.5
0
*
***
NS
***
Temperature (°C)
12 18 24 12 18 24 12 18 24 12 18 24
Cultivar
BT HE IM NV

C

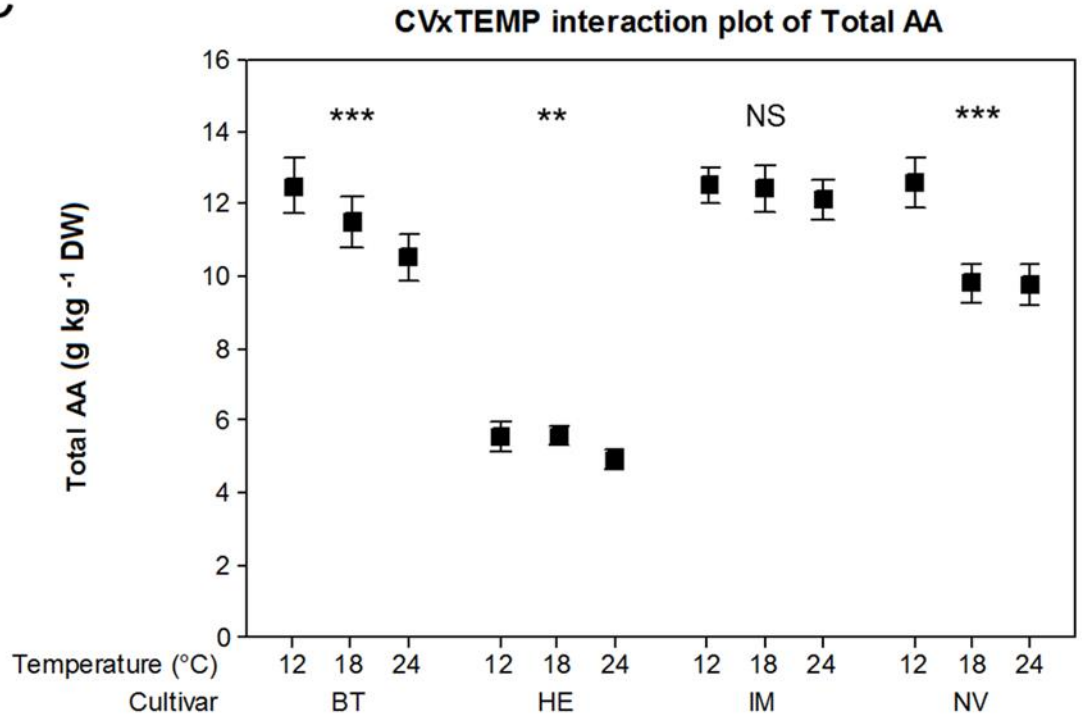


Figure 1. Cultivar x temperature interaction plots for (A) L-ascorbic acid, (B) dehydroascorbic acid and (C) total ascorbic acids. Data are the means of all daylength treatments, and the vertical bars are representing 95% confidence intervals for the means. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. Abbreviations used: BT = 'Ben Tron', HE = 'Hedda', IM = 'Imandra', NV = 'Narve Viking'.

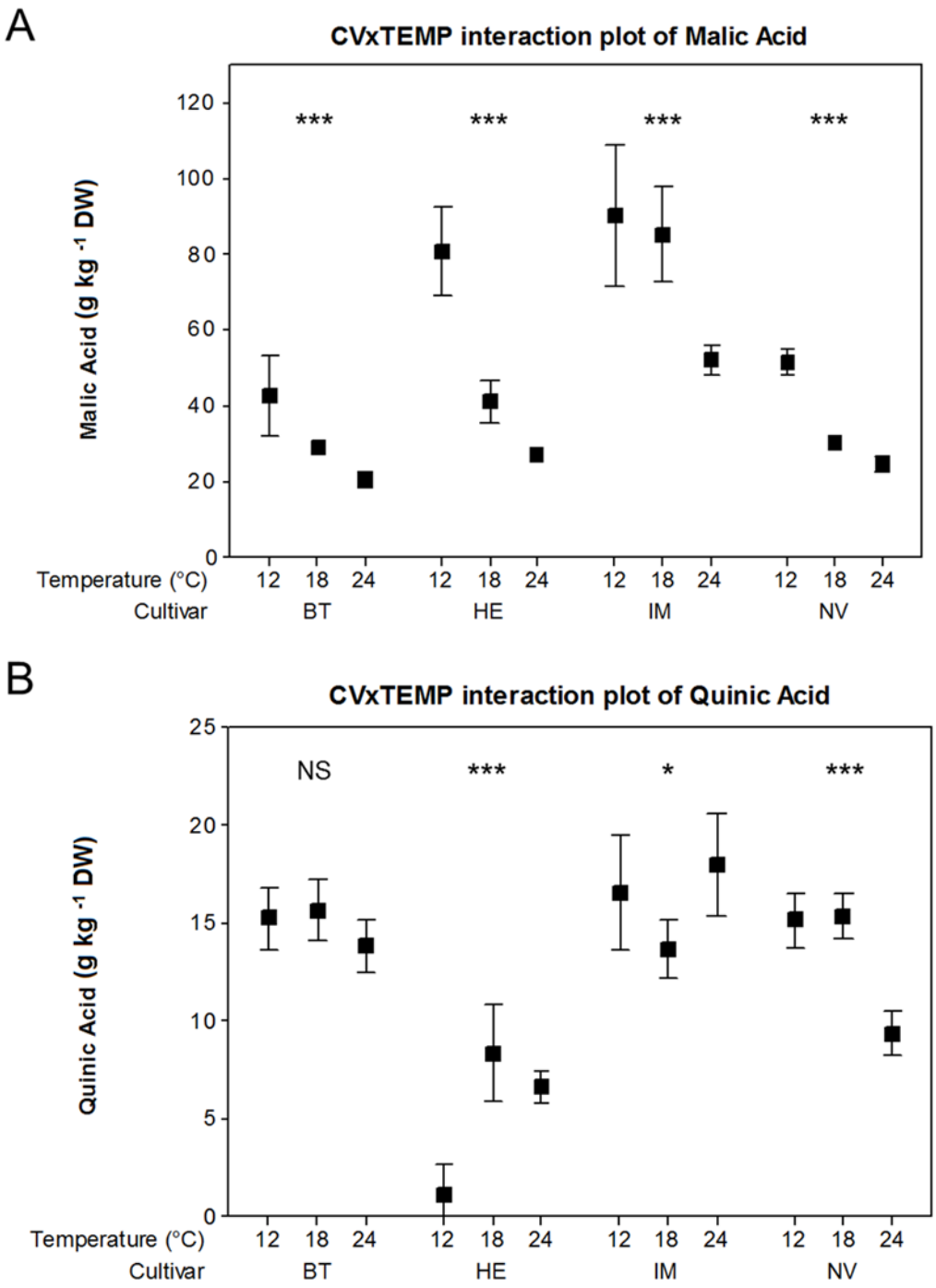


Figure 2. Cultivar x temperature interaction plots for (A) malic acid and (B) quinic acid. Data are the means of all daylength treatments, and the vertical bars are representing 95% confidence intervals for the means. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. Abbreviations used: BT = 'Ben Tron', HE = 'Hedda', IM = 'Imandra', NV = 'Narve Viking'.

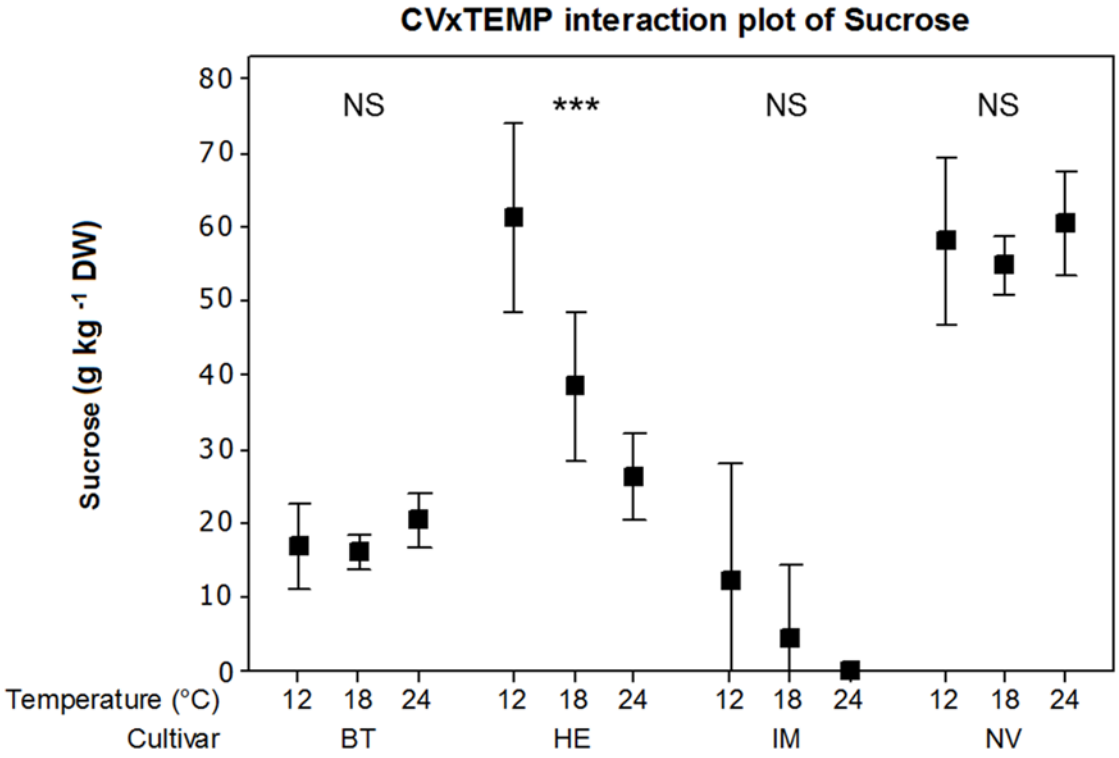


Figure 3. Cultivar x temperature interaction plots for sucrose. Data are the means of all daylength treatments, and the vertical bars are representing 95% confidence intervals for the means. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. Abbreviations used: BT = 'Ben Tron', HE = 'Hedda', IM = 'Imandra', NV = 'Narve Viking'.

Paper IV

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Influence of Controlled Postflowering Temperature and Daylength on Individual Phenolic Compounds in Four Black Currant Cultivars

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ABSTRACT: The effects of postflowering temperature and daylength on the concentration of individual phenolic compounds were studied in black currant (*Ribes nigrum* L.) berries under controlled phytotron conditions. The four cultivars studied varied greatly in their concentrations of individual phenolic compounds and temperature stability for accumulation. The concentrations of a wide range of identified phenolic compounds were strongly influenced by temperature over the 12−24 °C range, often with opposite temperature gradient patterns for compounds within the same subclass. Accumulation of anthocyanins and flavonols increased under natural long day conditions, which provided an increased daily light integral, while under identical light energy conditions, photoperiod had little or no effect on the concentration of phenolic compounds. Furthermore, with the exception of members of the hydroxycinnamic acid subclass, the concentration of most phenolic compounds was higher in berries ripened outdoors than in the phytotron, apparently due to screening of UV-B radiation by the glass cover.

KEYWORDS: *anthocyanins, black currant, climate, flavonols, hydroxycinnamic acids, light integral, photoperiod, Ribes nigrum, temperature*

INTRODUCTION

Phenolic compounds are among the most important and widespread secondary metabolites in plants. Classification of phenolic compounds is based mainly on the number of phenol rings (phenolic acids, stilbenes, flavonoids, lignans, and tannins). The flavonoids, mostly present as glycosides, are the main bioactive compounds found in fruits and can be divided into six subclasses: flavonols, flavonones, isoflavones, flavan-3-ols, flavones, and anthocyanins.[1]

According to Slimestad and Solheim (2002),[2] berries of black currant may contain up to 15 anthocyanin structures: the 3-*O*-glucosides and the 3-*O*-rutinosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin, cyanidin 3-*O*-arabinoside, and the 3-*O*-(6″-*p*-coumaroylglucoside)s of cyanidin and delphinidin. However, only four compounds are responsible for more than 97% of total anthocyanin content in black currant (3-*O*-glucosides and the 3-*O*-rutinosides of delphinidin and cyanidin). Black currant berries also contain flavonol glycosides and phenolic acids (cinnamic acid derivatives),[3] as well as proanthocyanins.[4]

Phenolic compounds are responsible for many of the positive, health-supporting effects of black currants[5] and influence the majority of characteristic sensory properties of black currant berries.[6,7] Anthocyanins from black currant had a positive effect on the cardiovascular system.[8] Possible anticancer properties of black currant constituents were also reported and rewieved by Folmer et al. (2014).[9] The pulmonary system can be supported by activity of proanthocyanidins, which has potential ability to relieve inflammation.[10] Moreover, it has been observed by clinical trials that black currant consumption can have positive effects on vision.[11,12] Black currant juice is also reported to improve mood and memory and to affect the monoaminooxidase (MAO) enzyme in humans, also due to the activity of phenolic compounds.[13] Anthocyanins of black currant can also have phytoestrogenic acivity,[14] as well as the ability to improve glucose metabolism.[15]

Biosynthesis and accumulation of phenolic compounds in black currant are influenced by genotype as well as environmental conditions.[3] Furthermore, anthocyanin concentration in black currant berries increases during the entire ripening period,[16] suggesting its evolutionary role as an antioxidant agent[17] and attractant of herbivores.[18] Accumulation of anthocyanins and other phenolic compounds in plants, which is mediated by phenylalanine ammonia-lyase (PAL) (catalyzes the transformation of phenylalanine to *trans*-cinnamic acid)[19] may be modified by light intensity and UV-B radiation levels.[20] According to numerous studies, production and accumulation of flavonoids in fruit crops is influenced by growth temperature.[3,20] Results from Sweden showed that total phenolic and anthocyanin content was higher in black currant berries from the southern (warmer) than from the northern locations.[21] However, different patterns of accumulation of phenolic compounds in black currant were also observed when comparing northern and southern European growing

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Table 1. Characterization of Phenolic Compounds in Black Currants Using HPLC-DAD-MS^{n}[a]

t_R (min)	λ_{max} (nm)	MW	MS (m/z)	MS^2 ions (m/z)[b]	MS^3 ions (m/z)[b]	tentative identification	abbreviations used	ref[c]
				Anthocyanins				
15.6	523	465	465 $[M]^+$	303	**257**, 229	delphinidin-3-glucoside	Dp-3-glu	2,29−31
17.2	525	611	611 $[M]^+$	465, **303**	**257**, 229	delphinidin-3-rutinoside	Dp-3-rut	2,29−31
18.6	516	449	449 $[M]^+$	287	**213,** 137	cyanidin-3-glucoside	Cy-3-glu	2,29−31
20.3	519	595	595 $[M]^+$	449, **287**	**213,** 137	cyanidin-3-rutinoside	Cy-3-rut	2,29−31
22.3	524	625	625 $[M]^+$	479, **317**	**302,** 274	petunidin-3-rutinoside	Pt-3-rut	2
24.0	507	579	579 $[M]^+$	433, **271**	197, **121**	pelargonidin-3-rutinoside	Pg-3-rut	2
26.6	518	609	609 $[M]^+$	463, **301**	**286**, 258	peonidin-3-rutinoside	Pn-3-rut	2
38.4	531	611	611 $[M]^+$	303	**257**, 229	delphinidin-3-(6″-coumaroyl)-glucoside	Dp-3-6-coumglu	2
40.3	523	595	595 $[M]^+$	287	**213,** 137	cyanidin-3-(6″-coumaroyl)-glucoside	Cy-3-6-coumglu	2
				Flavonols				
30.0	357, 263	626	625 $[M-H]^-$	607, **316**	271, 179	myricetin-3-rutinoside	M-3-rut	28−30
30.4	357, 261	480	479 $[M-H]^-$	316	271, 179	myricetin-3-glucoside	M-3-glu	28−30
33.6	357, 263	566	565 $[M-H]^-$	521	479, **316**	myricetin-3-malonylglucoside	M-3-malglu	28−30
			567 $[M+H]^+$	319	273, 165			
35.6	348, 256	610	609 $[M-H]^-$	301	**300**, 257, **179**, 151	quercetin-3-rutinoside	Q-3-rut	28−30
36.6	354, 256	464	463 $[M-H]^-$	301	**300**, 257, **179**, 151	quercetin-3-glucoside	Q-3-glu	28−30
39.0	357, 256	550	505 $[M-HCO_2]^-$[d]	463, **301**	**300**, 179, 151	quercetin-3-malonylglucoside	Q-3-malglu	28,29
			551 $[M+H]^+$	303	257, 229, 165			
39.3	355, 266	594	593 $[M-H]^-$	285	257, 229, 151	kaempferol-3-rutinoside	K-3-rut	28,29
				Hydroxycinnamic Acids				
10.1	324	354	353 $[M-H]^-$	**191**, 179, 135	173, 127, 111	caffeoylquinic acid		28,31
			355 $[M+H]^+$	163	145			
11.7	330	342	341 $[M-H]^-$	179	135	caffeoyl hexose		28,29,31
			707 $[2M+Na]^+$	365	185			
13.2	318	342	341 $[M-H]^-$	195, **163**	119	*p*-coumaric acid derivative 1		28
14.5	313	338	337 $[M-H]^-$	163	119	*p*-coumaroyl quinic acid		28
35.4	329	437	436 $[M-H]^-$	179	135	caffeic acid derivative		
			438 $[M+H]^+$	276	**163**, 114			
40.8	313	421	420 $[M-H]^-$	163	119	*p*-coumaric acid derivative 2		31
			422 $[M+H]^+$	**260**, 147	**147**, 114			
42.5	329	451	450 $[M-H]^-$	193	178, **149**, 134	ferulic acid derivative		29−31

[a]MS analyses were performed in both negative and positive mode. Results from both ionization modes are, however, only given when the identification was not straightforward. [b]The most abundant ions are shown in bold. These ions are isolated for fragmentation in MS^2. [c]Literature where the compound has been characterized by MS analysis. [d]$[M-H]^-$ was also detected, but not fragmented.

sites,[22,23] suggesting species adaptation ability to contrasting growing conditions.

In an eight-year study of weather impact on black currant chemical composition,[23] we found that, under field conditions in Southern Norway, the concentration of total monomeric anthocyanins and total phenolic compounds was negatively correlated with summer temperature. In addition, a positive correlation with summer precipitation was observed, but only for the total amount of phenolic compounds. This implies that simultaneous changes in various climatic factors, as commonly observed under field conditions, may complicate the proper interpretation of plant responses. Therefore, we wanted to investigate the effects of temperature and daylength on black currant quality under controlled environment conditions. In a recent paper[24] we reported that natural long day with increased light integrals enhanced the accumulation of total monomeric anthocyanins. Under controlled temperature conditions (12−24 °C range) an intermediate temperature of 18 °C was found to be optimal for anthocyanin accumulation.

High content of health-related compounds in berries in addition to balanced sensory characteristics is an important goal for food producers. A better understanding of the impact of environmental factors on the accumulation of these compounds may facilitate the improvement of production practices and, hopefully, the choice of black currant breeding strategies. The aim of the present study was thus to investigate the effects of controlled postflowering temperature and daylength conditions on the concentration of individual phenolic compounds in black currant berries.

MATERIALS AND METHODS

Plant Material and Cultivation. In our recent paper[24] the origin of the cultivars used, the raising and cultivation of the experimental plants, and the physical conditions during the experiment are explained in detail. In brief, the high-boreal Russian cultivar Imandra (IM), originating from the Kola Peninsula (67°30′N), Hedda (HE) and Narve Viking (NV) from the Norwegian breeding program at Ås (59°40′N), and Ben Tron (BT) from the Scottish breeding program in Dundee (56°30′N) were used because of their distinct genotypes and latitudinal differences of origin.[25] During the last 3 weeks of berry maturation, the plants were exposed to constant temperatures of 12, 18, and 24 °C (±1 °C) combined with the following photoperiodic conditions: (1) natural long summer daylight, ca. 19 h (natural LD), (2) 10 h summer daylight, short day (SD), and (3) 10 h SD + 3 h night interruption (SD + NI). Both treatments 1 and 3 were perceived as long day (LD) conditions by the plants, but the former also provided a 9% larger daily light integral (total daily photosynthetic

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Table 2. Effects of Cultivar, Temperature, and Daylength on Individual and Total Anthocyanins (μg/g DW) in Black Currant Berries[a]

	variables	Dp-3-glu[b]	Dp-3-rut	Cy-3-glu	Cy-3-rut	Pt-3-rut	Pg-3-rut	Pn-3-rut	Dp-3-6-coumglu	Cy-3-6-coumglu	total anthocyanins
cultivar effect	BT	1089 b	4380 b	362 a	2893 a	74 a	13 a	46 a	78 c	24 b	8959 a
	HE	840 d	3064 d	173 c	1469 c	66 b	1 c	24 b	116 a	22 c	5775 c
	IM	979 c	4866 a	292 b	2939 a	34 d	5 b	14 d	105 b	34 a	9269 a
	NV	1215 a	3715 c	304 b	1904 b	44 c	4 b	19 c	46 d	6 d	7258 b
	significance[c]	***	***	***	***	***	***	***	***	***	***
temperature effect	12 °C	828 c	3783 b	314 a	2601 a	53 a	6 b	29 a	42 c	16 c	7669 b
	18 °C	1039 b	4713 a	268 b	2450 a	58 a	8 a	25 b	89 b	24 b	8674 a
	24 °C	1228 a	3523 b	266 b	1852 b	54 a	3 c	24 b	127 a	25 a	7103 c
	significance	***	***	***	***	NS	***	***	***	***	***
daylength effect	natural LD	1088 a	4170 a	295 a	2364 a	56 ab	6 a	26 ab	91 a	23 a	8119 a
	SD	996 b	3854 b	276 a	2232 a	52 b	5 a	25 b	83 b	21 b	7544 b
	SD + NI	1007 ab	3995 ab	277 a	2308 a	57 a	6 a	27 a	84 b	21 b	7782 ab
	significance	*	*	NS	NS	*	NS	*	**	*	*
	CVxTEMP	***	***	**	**	NS	***	***	***	***	***
	CVxDAY	**	*	*	*	*	NS	NS	**	*	*
	TEMPxDAY	NS	*	NS	NS	NS	NS	NS	**	*	NS
	CVxTEMPxDAY	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	ambient control	1669	4967	419	2810	69	9	30	174	45	10190

[a]All data are means based on four replicates. [b]Means that do not share a letter are significantly different at the $p < 0.05$ level, with comparisons performed using Tukey's test. [c]Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see Materials and Methods and Table 1.)

active radiation). On the other hand, by using low intensity incandescent lamps for the night interruption (approximately 7 μmol quanta m^{-2} s^{-1}), the daily light integral varied by less than 0.5% between treatments 2 and 3, which represent the true photoperiodic test. Plants of all cultivars were also grown outdoors in pots (as a control) under ambient summer conditions (59°40′N). Berries were hand harvested when fully ripe as judged by berry softness and visual assessment of color. Harvested berries were immediately frozen and stored at −20 °C until analyzed.

Experimental Design and Statistical Analysis. The experiment was fully factorial with a split-plot design, with temperatures as main plots and photoperiod and cultivar as subplots. The experiment was replicates with four randomized blocks, each comprising two plants of each cultivar on a separate trolley, giving eight plants per treatment. One berry sample (200−500 g) of each cultivar from each plant treatment replicate was analyzed. A three-factor fixed effect model was used to analyze the data. Effects of cultivar (CV, fixed effect, four levels), temperature (TEMP, fixed effect, three levels), and daylength conditions (DAY, fixed effect, three levels) were analyzed by a general linear model (GLM) and Tukey's multiple comparison test with significance levels $\alpha = 0.05$. Results showing significant interactions between cultivar and temperature indicating differences in the cultivar responses are presented together with bars representing 95% confidence intervals (CI). Treatment results were considered as significantly different if the confidence interval of one did not overlap the other.[26] Additional decomposition and one-way ANOVA tests within cultivars (differences between temperatures) were also performed. Principal component analysis (PCA) was used to assess the relation between cultivars and fruit chemical composition. All calculations were performed using a Minitab 16 Statistical Software Package (Minitab Inc., State College, PA, USA). To avoid any possible dilution biases, data are presented on a dry weight (DW) basis.[27]

Chemicals Used. Cyanidin-3-glucoside was obtained from Polyphenols AS (Sandnes, Norway). Quercetin-3-rhamnosylglucoside (rutin) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chlorogenic acid and formic acid (LC−MS grade) were obtained from Fluka (Buchs, Switzerland). Acetonitrile (HPLC grade) was obtained from VWR International (Fontenay-sous-Bois, France), and water was of Milli-Q quality (Millipore Corp., Bedford, MA, USA).

Extraction of Phenolic Compounds. Black currant berries (30 g) were homogenized with a blender (Braun MR400, Karlsruhe, Germany), and an aliquot of the homogenate (3 g) was extracted with 1 mM HCl (37%) in methanol (30 mL). The samples were flushed with nitrogen, capped, and vortexed (Vortex-T Genie 2, Scientific Industries Inc., Bohemia, NY, USA), followed by sonication at 0 °C for 15 min (Bandelin SONOREX RK 100, Bandelin Electronic GmbH & Co., Berlin, Germany). After centrifugation, the liquid samples were stored at −20 °C until analyzed.

Analysis of Phenolic Compounds by HPLC-DAD-MS^n. Extract of phenolic compounds was filtered through a Millex HA 0.45 μm filter (Millipore Corp., Billerica, MA, USA) before analysis on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler cooled to 4 °C, a diode array detector, and an MSD XCT ion trap mass spectrometer fitted with an electrospray ionization interface as previously described.[27] Chromatographic separation was performed on a Synergi 4 μm MAX RP C12 column (250 mm × 2.0 mm i.d.) equipped with a 5 μm C12 guard column (4.0 mm × 2.0 mm i.d.), both from Phenomenex (Torrance, CA, USA), with mobile phases consisting of A, formic acid/water (2/98, v/v), and B, acetonitrile. The phenolic compounds were identified based on their UV−vis spectra (220−600 nm), mass spectra and retention times relative to external standards, and comparison with previous reports on phenolic compounds in black currants.[2,28−31] The phenolic compounds were classified based on their characteristic UV−vis spectra and quantified by external standards. Anthocyanins were quantified as cyanidin-3-glucoside at 520 nm, flavonols as rutin at 360 nm, and hydroxycinnamic acid derivatives as chlorogenic acid 320 nm. All results were expressed as μg per g DW.

RESULTS

Retention times, spectral characteristics, tentative identification, and abbreviations of the phenolic compounds quantified in the present study are shown in Table 1. The flavonoids in the berries were mainly glucosides and rhamnosylglucosides (rutinosides) of anthocyanins and flavonols with two or three

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Table 3. Effects of Cultivar, Temperature, and Daylength on Individual and Total Flavonols (μg/g DW) in Black Currant Berries[a]

	variables	M-3-rut[b]	M-3-glu	M-3-malglu	Q-3-rut	Q-3-glu	Q-3-malglu	K-3-rut	total flavonols
cultivar effect	BT	239 b	312 a	78 b	155 b	435 a	75 a	36 b	1329 a
	HE	249 ab	208 b	74 b	199 a	297 b	64 b	42 b	1133 b
	IM	264 a	323 a	90 a	143 c	101 d	75 a	0 c	995 c
	NV	237 b	169 c	40 c	112 d	175 c	34 c	60 a	827 d
	significance[c]	***	***	***	***	***	***	***	***
temperature effect	12 °C	222 b	181 c	77 a	160 a	256 a	72 a	37 a	1006 c
	18 °C	298 a	260 b	73 b	154 a	256 a	62 b	33 b	1137 a
	24 °C	221b	317 a	62 c	142 b	242 a	52 c	33 b	1070 b
	significance	***	***	***	**	NS	***	*	***
daylength effect	natural LD	266 a	269 a	72 a	154 a	266 a	61 a	33 a	1120 a
	SD	234 b	247 b	69 a	149 a	245 b	62 a	34 a	1036 b
	SD + NI	241 b	243 b	70 a	149 a	245 b	63 a	36 a	1057 b
	significance	***	***	NS	NS	*	NS	NS	***
	CVxTEMP	***	***	NS	***	***	***	**	***
	CVxDAY	NS	**	**	*	*	**	NS	**
	TEMPxDAY	*	**	NS	NS	NS	NS	NS	*
	CVxTEMPxDAY	NS	NS	NS	NS	NS	NS	NS	NS
	ambient control	303	401	83	158	214	40	55	1254

[a]All data are means based on four replicates. [b]Means that do not share a letter are significantly different at the $p < 0.05$ level, with comparisons performed using Tukey's test. [c]Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see Materials and Methods and Table 1.)

Table 4. Effects of Cultivar, Temperature, and Daylength on Individual and Total Hydroxycinnamic Acids (μg/g DW) in Black Currant Berries[a]

	variables	caffeoyl quinic acid[b]	caffeoyl hexose	*p*-coumaric acid derivative 1	*p*-coumaroyl quinic acid	caffeic acid derivative	*p*-coumaric acid derivative 2	ferulic acid derivative	total hydroxycinnamic acids
cultivar effect	BT	612 a	346 b	99 b	126 b	121 c	256 a	67 b	1627 a
	HE	113 d	318 c	52 d	87 c	185 a	239 ab	32 c	1026 c
	IM	151 c	241 d	64 c	125 b	41 d	62 c	22 d	706 d
	NV	347 b	401 a	166 a	149 a	144 b	232 b	75 a	1515 b
	significance[c]	***	***	***	***	***	***	***	***
temperature effect	12 °C	323 a	392 a	118 a	141 a	107 c	228 a	53 a	1361 a
	18 °C	294 b	317 b	99 b	124 b	123 b	188 b	49 b	1194 b
	24 °C	301 b	270 c	69 c	100 c	139 a	176 b	46 c	1101 c
	significance	**	***	***	***	***	***	***	***
daylength effect	natural LD	295 a	336 a	95 a	121 a	123 a	191 a	47 b	1208 a
	SD	309 a	327 ab	95 a	123 a	121 a	199 a	50 ab	1224 a
	SD + NI	313 a	316 b	96 a	122 a	124 a	202 a	51 a	1224 a
	significance	NS	*	NS	NS	NS	NS	**	NS
	CVxTEMP	***	***	***	***	***	**	***	***
	CVxDAY	NS	NS	NS	*	NS	NS	NS	*
	TEMPxDAY	NS	NS	NS	NS	NS	NS	NS	NS
	CVxTEMPxDAY	NS	NS	*	NS	*	NS	NS	NS
	ambient control	270	252	76	103	99	153	43	996

[a]All data are means based on four replicates. [b]Means that do not share a letter are significantly different at $p < 0.05$ level, with comparisons performed using Tukey's test. [c]Levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see the Materials and Methods section and Table 1).

hydroxyl groups on ring B, that is, cyanidin/quercetin and delphinidin/myricetin, respectively. Seven compounds were assigned as hydroxycinnamic acids based on their UV spectra and MS fragmentation pattern. The structures of the three late eluting compounds with molecular weights (MW) 437, 421, and 451 were difficult to elucidate. The unusual odd molecular weights indicates that these compounds contain nitrogen. Further, they shared the common features with loss of unknown masses of 257 amu in negative mode and 113 amu in positive mode. The compounds had MS^2 fragments in negative mode at m/z 179, 163 and 193, with a consecutive loss of 44 (CO_2) in MS^3, suggesting that the compounds contained caffeic, coumaric, and ferulic acid, respectively. Further, all compounds had loss of 162 amu in negative mode, which indicates the presence of hexose, and the compounds were thus tentatively identified as hexose derivatives of caffeic acid, *p*-

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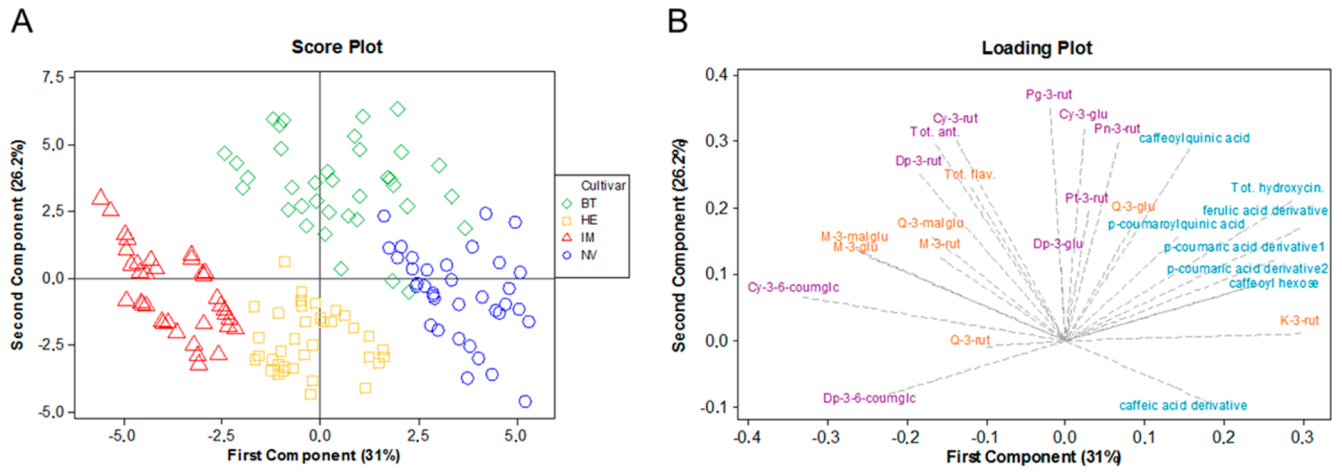

Figure 1. Principal component analysis (PCA) based on the correlation matrix of all the samples. Score plot (A) and loading plot (B).

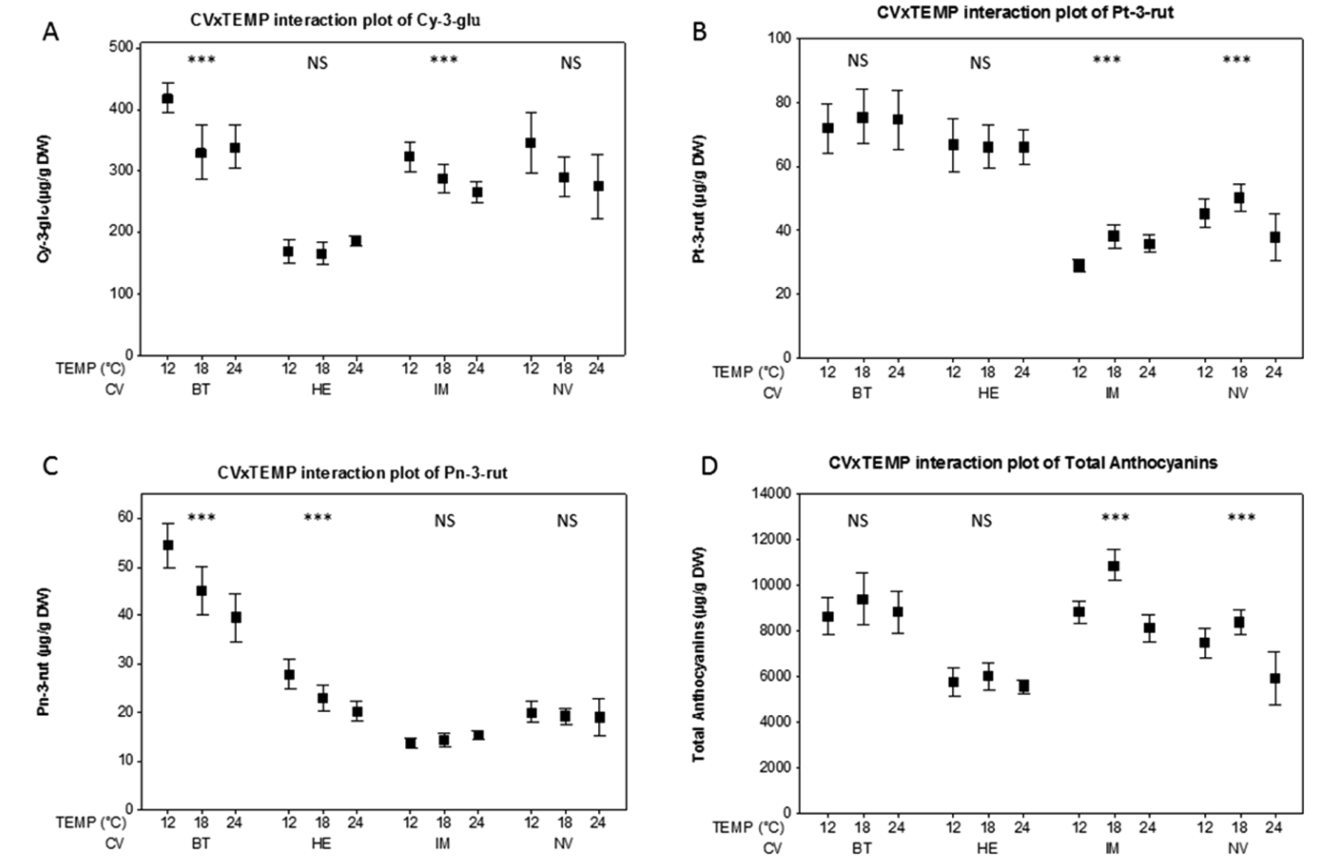

Figure 2. Cultivar × temperature interaction plots for Cy-3-glu (A), Pt-3-rut (B), Pn-3-rut (C), and total anthocyanins (D). Data are the means of all daylength treatments, and the vertical bars represent 95% confidence intervals for the means. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see Materials and Methods and Table 1.)

coumaric acid, and ferulic acid. For more complete identification of these less polar hydroxycinnamic acid derivatives, NMR analysis is needed.

The genetic background of the investigated cultivars had highly significant ($p < 0.001$) influence on the content and proportion of all phenolic compounds quantified in the berries (Tables 2, 3, and 4). PCA was applied to visualize the general differences in berry content of phenolic compounds among the investigated cultivars (Figure 1A,B). The first two principal components represented 31 and 26% of the variance, respectively, with a total variance of 57%. Score plots of the first versus second component of the PCA model showed a clear separation of the four cultivars studied (Figure 1A). The corresponding loading plot (Figure 1B) establishes the relative amounts of each compound for the cultivars presented in Figure 1A. Berries of Ben Tron were characterized mainly by high concentrations of anthocyanins, except the coumaroyl glucosides as well as high total flavonol concentrations. Rutin (Q-3-rut) showed the strongest affiliation to cultivar Hedda, while Imandra was characterized by high concentrations of Cy-

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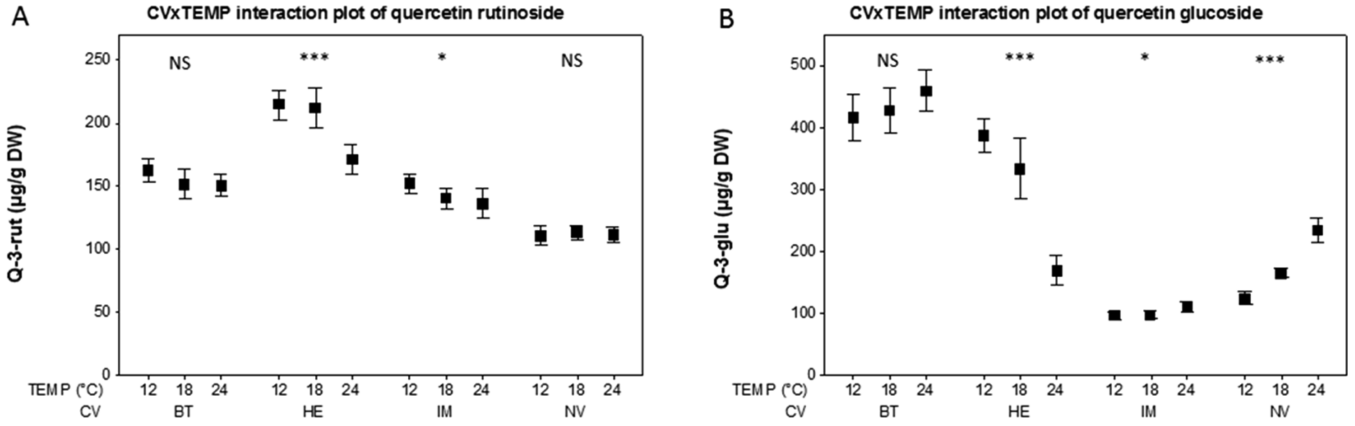

Figure 3. Cultivar × temperature interaction plots for quercetin rutinoside (A) and quercetin glucoside (B). Data are the means of all daylength treatments, and the vertical bars represent 95% confidence intervals for the means. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see Materials and Methods and Table 1.)

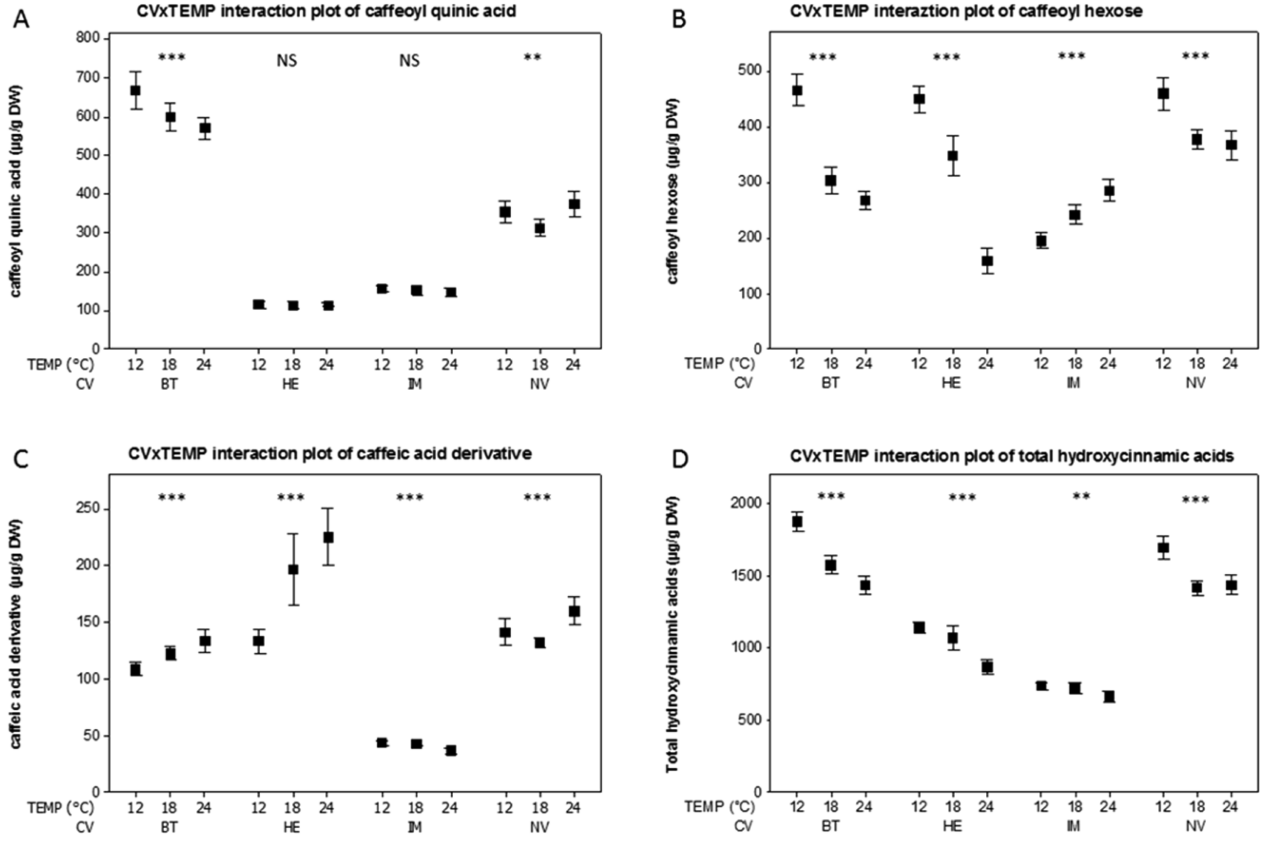

Figure 4. Cultivar × temperature interaction plots for caffeoyl quinic acid (A), caffeoyl hexose (B), caffeic acid derivative (C), and total hydroxycinnamic acids (D). Data are the means of all daylength treatments, and the vertical bars represent 95% confidence intervals for the means. Symbols on the top of each panel indicate the results of Tukey's test for each cultivar. Tukey's test levels of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = not significant. (For abbreviations, see Materials and Methods and Table 1.)

3-6-coumglu, as well as M-3-glu and M-3-malglu. Narve Viking showed the strongest relation to K-3-rut and hydroxycinnnamic acids. The loading plot (Figure 1B) also gives additional information about the relationships between the investigated compounds. Compounds that are close to each other on the plot denote a strong positive correlation, while a strong negative correlation is denoted by compounds that are symmetrically distant on the loading plot area.

The most abundant anthocyanins in black currant berries were Dp-3-rut, followed by the Cy-3-rut, Dp-3-glu, and Cy-3-glu (Table 2). The cultivars Imandra and Ben Tron had the highest concentration of total anthocyanins. Increased temperature over the 12−24 °C range caused a significant increase in the concentrations of Dp-3-glu, Dp-3-6-coumglu, and Cy-3-6-coumglu, while the opposite trend, where the coolest ripening conditions caused an increased accumulation, was observed for Cy-3-glu, Cy-3-rut, and Pn-3-rut. However, decomposed data

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showed that Hedda had the most stable (and lowest) accumulation of Cy-3-glu over the 12−24 °C range (Figure 2A). Also, stable (and high) accumulation of Pt-3-rut was observed for Ben Tron and Hedda (Figure 2B). The concentration of Pn-3-rut was not significantly affected by ripening conditions in berries of Imandra and Narve Viking (Figure 2C). The general pattern of accumulation of Dp-3-rut, the most abundant anthocyanin in black currant, and thus also for total anthocyanins, where a temperature of 18 °C was found to be optimal, revealed little effect of temperature in cultivars Ben Tron and Hedda (Figure 2D). In berries matured under ambient outdoor conditions, the concentrations of all individual anthocyanins were markedly higher than those observed in the phytotron (Table 2). Compared with SD, natural summer daylength conditions promoted accumulation of Dp-3-glu, Dp-3-rut, Dp-3-6-coumglu, and Cy-3-6-coumglu as well as total anthocyanins. Under identical light energy conditions (SD vs SD + NI), daylength had no significant effect on the concentrations of the predominating and total anthocyanins (Table 2).

In all the studied black currant cultivars, M-3-rut, M-3-glu, and Q-3-glu were the predominant flavonols (Table 3). Whereas Ben Tron was found to be the richest in flavonols, Narve Viking and Imandra had the lowest concentrations of flavonols among the studied cultivars. Interestingly, K-rut was not detected in cultivar Imandra. High ripening temperature promoted accumulation of M-3-glu, while M-3-malglu and Q-3-malglu showed the opposite trend. For M-3-rut and total flavonols, the highest concentrations were found at 18 °C. Although temperature did not have any significant main effect on the concentration of Q-3-glu, decomposition of the data showed highly variable responses among the cultivars, with a large reduction at elevated temperature in Hedda (Figure 3B). As for the anthocyanins, the concentrations of total and the majority of the predominant flavonols were higher in berries ripened under ambient outdoor conditions than in those from the controlled environment trial. M-3-rut, M-3-glu, and Q-3-glu, as well as total flavonols, all increased significantly under natural LD conditions compared with the SD or SD + NI treatments (Table 3).

A caffeoyl quinic acid and a caffeoyl hexose were the most abundant hydroxycinnamic acid derivatives detected in the black currant berries, and Ben Tron was the cultivar with the highest total concentration of these compounds (Table 4). Accumulation of the majority of the hydroxycinnamic acid derivatives was promoted by low ripening temperature across the entire temperature range. The only exception was a derivative of caffeic acid that showed the opposite trend (Table 4). However, there were highly significant cultivar × temperature interactions for all the compounds. The cultivar × temperature interaction plots in Figure 4 illustrate marked differences in the cultivars' temperature stability for the accumulation of the various compounds, sometimes even with opposite temperature gradient patterns for the same substance. Whereas a highly significant low temperature enhancement of the accumulation of caffeoyl quinic acid was observed only in Ben Tron (Figure 4A), the low temperature enhancement effect was highly significant in all cultivars for the caffeoyl hexose, and total hydroxycinnamic acids (Figure 4B,D).The deviating temperature response of Imandra was particularly marked for the accumulation of caffeoyl hexose (Figure 4B). Imandra also had remarkable temperature stability for the accumulation of the other hydroxycinnamic acids (Figure 4A,C,D). In contrast to the situation for the other phenolic compounds in black currant berries, the concentration of hydroxycinnamic acids was lower rather than higher in berries ripened outdoors in comparison with glasshouse conditions (Table 4). Photoperiod had no significant effect on the concentration of hydroxycinnamic acids in the studied black currant cultivars.

DISCUSSION

The identity and quantity of phenolic compounds reported in the present study were in general accordance with previous findings in black currants.[2,28−31] The identity of three less polar hydroxycinnamic acids was only tentatively determined. However, previous studies similarly reported three late eluting compounds that were assigned as glucose derivatives of caffeic, *p*-coumaric, and ferulic acid, respectively.[32,33]

The present results confirm the importance of plant genotype as a main factor influencing the concentration of phenolic compounds in black currant berries.[3,21] However, this may at least in part be an indirect effect of differences in berry size. Most abundant phenolic compounds in black currants (anthocyanins and flavonols) are found in the berry skin. Small berries have a higher skin to volume ratio, and therefore, cultivars with small berries will also have higher concentrations of phenolic compounds.[34]

Temperature and light intensity are the major environmental factors influencing the concentration of phenolic compounds in plants grown in natural environments.[20] The results of our experiment were in general agreement with this statement, also for black currants ripened under controlled environment conditions. Yamane et al. (2006)[35] also observed that moderate temperature (20 °C) during ripening enhanced accumulation of anthocyanins in grape berry skins compared with the situation at 30 °C. Moreover, a strong reduction of anthocyanin accumulation under 35 °C heat stress was observed in grapes,[36] as a result of both anthocyanin degradation and inhibition of mRNA transcription. It is also known that low temperature during ripening of apples can be an inducing factor for the expression of key genes controlling anthocyanin biosynthesis, such as CHS (chalcone synthase), ANS (anthocyanidin synthase), and UFGluT (UDP-glucose:flavonoid 3-*O*-glucosyltransferase).[37]

However, our results with black currants demonstrated differential temperature responses for the various anthocyanins. An increasing concentration of Dp-3-glu with increasing temperature as found in the present study (Table 2) is in agreement with previous observations from Finland, where the concentration of this anthocyanin in black currant berries showed a positive correlation with summer temperature.[3] The same experiment also revealed an enhancement of the concentration of Dp-3-rut at more southern locations in Finland, while our observations showed that accumulation of this anthocyanin had an optimum at intermediate temperature (18 °C). Similar studies conducted in Sweden[21] confirm the patterns of accumulation of Dp-3-glu and Cy-3-rut observed in the present experiment.

An additional 9% increase in the daily light energy supply as received by the plants grown under natural LD conditions resulted in an enhanced accumulation of several anthocyanins. That may be explained by an upregulation of flavonoid-related biosynthesis genes by light as described by Azuma et al. (2012).[38] The crucial photoperiodic treatments, on the other hand, had only marginal influence of anthocyanin accumulation

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(Table 2). It should be noticed that, under identical light integrals, there was no clear separation of the results for the true photoperiodic treatments (SD vs SD + NI), either for any specific anthocyanin or for their sum. On the other hand, variation in anthocyanin composition among the four cultivars and the highly significant interaction of cultivar × temperature in the accumulation of these compounds (Table 2) demonstrate that the effect of temperature on anthocyanin accumulation varies considerably between the cultivars. These issues should be studied in further investigations with cultivars grown under fluctuating day/night temperatures.

Large variation between cultivars in flavonol contents as revealed by the results in Table 3 was observed also by other authors.[39] The assumed temperature effects of field cultivation of black currant cultivars in Southern and Northern Sweden[21] showed mostly opposite temperature patterns of accumulation for this group of flavonoids compared with our findings under controlled conditions. It should be kept in mind, however, that other cultivars were used in the two studies and that, under outdoor conditions, several interrelated factors can vary simultaneously, and thus incur complicating covariations. Furthermore, our study also demonstrated highly significant cultivar × temperature interactions in the accumulation of flavonols. On the other hand, Zheng et al. (2012)[3] showed that M-3-glu and Q-3-glu as well as the total flavonol glycosides were all positively correlated with temperature and radiation during ripening, results that are in general agreement with our results under controlled conditions (Table 3). Accumulation of Q-3-glu over the 12−24 °C temperature range revealed different and sometimes contrasting temperature responses among the investigated cultivars, suggesting specific differences in the gene pools of the various breeding programs. Imandra and related high-latitude cultivars seemed to be genetically distinct from most European commercial cultivars in growth and flowering characteristics,[25,40] and the present PCA data further indicate that this was the case also for berry composition of phenolic compounds (Figure 1A, B).

Expression of the gene encoding flavonol synthase (FLS) in grapes was greatly downregulated under shade conditions leading to a 10% reduced flavonol accumulation.[41] This mechanism may have played a role in the decreasing accumulation of some flavonols observed in our experiment under decreased daily light integral prevailing under SD conditions (Table 3).

The observed impact of environmental factors on the accumulation of hydroxycinnamic acids in black currant berries (Table 4) is in general agreement with previous studies. The total concentration of these compounds was found to be higher in berries produced in more northern (colder) parts of Finland for two local black currant cultivars, but not for a third one.[3] The content of hydroxycinnamic acid derivatives in the green black currant cultivar Vertti and the white currant White Dutch grown in northern Finland was also 30% higher than in those grown in the southern locations.[33] These results are in agreement with the present finding that accumulation of hydroxycinnamic acids in general was markedly enhanced by low temperature (Table 4), although some cultivars showed higher temperature stability than others (Figure 4). Moreover, some exceptions were also observed for specific compounds such as the unidentified caffeic acid derivative. In contrast to the situation for anthocyanins and flavonols, the concentrations of individual and total hydroxycinnamic acid derivatives were lower in berries ripened outdoors than under phytotron conditions (Table 4). This is an interesting observation that may be related to specific differences in the biosynthetic pathways of these compounds (see below).

Generally, accumulation of anthocyanins and flavonols seems to be mediated by UV-B radiation by influencing the phenylalanine ammonia-lyase enzyme activity, a key enzyme in the flavonoid biosynthesis pathway.[20] It is also well documented that UV-B absorbing flavonoids accumulate in epidermal cells as a protection mechanism against the damaging effect of UV-B radiation.[42] Therefore, one of the reasons for lower concentration of the flavonoids in berries matured in the phytotron, compared with outdoor conditions (Table 2 and 3), might be an almost complete screening of UV-B radiation by a glass cover. The absence of a similar response for the accumulation of the hydroxycinnamic acids (Table 4) might possibly be due to a strong UV-B upregulation reported for the chalcone synthase gene (CHS) encoding the CHS enzyme that is acting specifically on the flavonoid biosynthesis pathway downstream of the general phenylpropanoid pathway (refs 20 and 43 and references therein).

Furthermore, anthocyanin biosynthesis in apple skin was shown to be sensitive to low night temperature, a single night chilling temperature being sufficient to upregulate the transcription of the MYB10 factor and enhance anthocyanin biosynthesis.[44] This mechanism might have been an additional reason for the lower anthocyanin accumulation under phytotron conditions, where ripening took place under constant temperatures without night chilling. Nevertheless, the concentrations of the various phenolic compounds found in berries of plants grown in the phytotron were only slightly lower than those reported for field experiments,[21] and outdoors in the present experiment. Further studies under controlled environment conditions are now on the way to directly assess the effect of fluctuating day/night temperature.

In conclusion, the presented results confirm the previously reported finding,[3,21,24] that black currant cultivars vary strongly in berry chemical composition. With the exception of hydroxycinnamic acids, the concentration of phenolic compounds was higher in berries ripened outdoors than in the phytotron, apparently due to different responsiveness to screening of UV-B radiation by the glass cover. Generally, the present and previous results[3,21,23,24] indicate that cool temperature conditions and relatively high radiation are favorable for production of black currant berries with good taste and high content of health promoting substances. On the other hand, photoperiod had no significant effect on the prevalence of phenolic compound in black currant berries. Varying temperature stability for the accumulation of specific compounds, as observed among cultivars from different breeding programs, is an interesting observation that may be of importance for future black currant breeding strategies.

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Notes

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