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Effects of Post-Flowering Environmental Variation along an Altitudinal Gradient on Chemical Composition of 'Glen Ample' Red Raspberry (*Rubus idaeus* L.)

S. P. Mazur[1), 2)], A. Sønsteby[1)], A. Nes[1)], A. B. Wold[2)], A. Foito[3)], S. Freitag[3)], S. Verrall[3)], D. Stewart[3)] and O. M. Heide[4)]

([1)]Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Ås, Norway, [2)]Department of Plant Sciences, Norwegian University of Life Sciences, Ås, Norway, [3)]Environment and Biochemical Sciences, James Hutton Institute, Invergowrie, UK and [4)]Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway)

Summary

The effects of post-flowering environmental conditions on chemical composition of 'Glen Ample' red raspberry were studied by growing plants at three altitudes (128, 340 and 670 m a. s. l.) at the same latitude in central South Norway. Due to day-to-day variation in temperature and precipitation, the average climatic effects over the entire harvest period were evened out and reduced in magnitude, thus complicating the analysis of their effects. However, correlation of fruit chemical composition for the individual weekly harvests with temperature and precipitation data for the current and preceding weeks, revealed significant effects of weather conditions during fruit maturation. At the lower temperature range established in the present experiment, fruit quality attributes such as dry matter, soluble solids, titratable acids, total monomeric anthocyanins, and total phenolic concentration were all positively correlated with increasing temperature during the final two weeks of fruit maturation, while precipitation during the same period had the opposite effect. It is concluded, however, that the negative effect of precipitation was not likely to be a direct plant-water relation effect, but rather an indirect effect related to increased cloudiness and reduced radiation during rainy weather. Specific effects on, and interrelations between individual compounds were observed and discussed.

Key words. fruit quality – growth temperature – anthocyanins – antioxidant capacity – ascorbic acid – polyphenols

Introduction

Red raspberry (*Rubus idaeus* L.) is an economically important small fruit crop that is highly valued for the flavour and nutritional quality of its fruit. Growing consciousness about the health beneficial properties of a range of chemical constituents in raspberry fruit has increased its value considerably. The impact of environmental factors such as temperature, day-length, light quality and intensity, and precipitation on fruit chemical composition and sensorial quality has been widely studied in red raspberry as well as in other berry species and cultivars (Connor et al. 2002; Moore et al. 2005; Zheng et al. 2009; Tulipani et al. 2011; Krüger et al. 2012). However, because of confounding effects of interacting environmental factors, interpretation of experimental data from open field experiments can be difficult. Therefore, more effort has recently been placed on experimentation in controlled environments where the effects and interactions of the various environmental factors on fruit quality can be better assessed (Remberg et al. 2010; Uleberg et al. 2012; Mazur et al. 2014).

In such a controlled environment experiment with long cane plants of 'Glen Ample' raspberry, it was found that fruit weight and moisture content decreased, while the concentration of several bioactive compounds increased significantly with increasing post-flowering temperature in the 12 °C to 24 °C range, and with the progress of harvest (Remberg et al. 2010). The authors concluded that these effects were mainly dilution effects related to fruit size and moisture content. The concentrations of vitamin C, cyanidin-3-sophoroside and cyanidin-3-xylosylrutino-

side, however, increased with decreasing temperature, even on a dry weight basis. In a recent study, MAZUR et al. (2014) demonstrated significant and contrasting effects of photoperiod on fruit concentrations of a range of bioactive compounds in the same red raspberry cultivar. Total phenolics (TP), L-ascorbic acid (L-AA), organic acids, and the antioxidant capacity (AOC), all increased significantly when expressed on dry weight (DW) basis, while the concentration of reducing sugars decreased in fruits matured under long day (LD) compared with short day (SD) conditions. The same effects were obtained whether LD was provided by day-length extension or by night interruption. It was, therefore, concluded that the observed changes in nutritional quality of raspberry fruit were mediated through specific photoperiodic mechanism(s) independently of daily light energy supply.

Because controlled environment facilities that can accommodate experiments with large fruiting berry bushes are costly and not widely available, such experiments are still rather scarce. Therefore, alternative strategies have been attempted to circumvent or reduce the problems of co-variation among environmental factors under natural outdoor conditions. One such approach is to grow plants along an altitudinal gradient in order to establish a temperature gradient under similar day-length and light conditions. This was successfully done by BRADISH et al. (2011) in order to study day and night temperature effects on flavonoid composition of autumn-fruiting red raspberry cultivars in Southern United States. They reported that variation in flavonoid composition was mainly attributed to genotype, but was also associated with night temperature and hours of exposure to temperatures over 29 °C.

Here we report the results of a similar experiment with 'Glen Ample' raspberry grown at three altitudinal locations in central South Norway, all at latitude approximately 61° N. Since in such experiments, it is important to limit the time the plants are exposed to the experimental conditions as much as possible in order to avoid long-term carry-over effects, pot grown plants were used and exposed to the respective experimental conditions only during fruit development and maturation.

Materials and Methods

Plant Material and Cultivation

Pot-grown long cane red raspberry (*Rubus idaeus* L.) plants cv. 'Glen Ample' were produced as described earlier (SØNSTEBY et al. 2009). After overwintering in a cold store at −2 °C, the plants were tipped at 160 cm height, transplanted into 7.5 L pots on April 24, 2011, and placed in a polyethylene tunnel with minimum temperature of 15 °C and natural light conditions at the Bioforsk Experimental Centre Apelsvoll, Kapp, Norway (60° 40' N, 10° 52' E). The plants were fertilized three times weekly with a complete fertilizer solution with electric conductivity of 1.5 mS cm^{-1} via an automatic drip irrigation system. When the plants had started flowering and reached the developmental stage of first unripe fruit shown by REMBERG et al. (2010), they were divided into three groups and were moved to the following three altitudinal experimental sites in central South Norway: Kise (128 m a. s. l.), Næroset (340 m a. s. l.) and Kvarstad (670 m a. s. l.) on July 4th (week 27) 2011. The sites were selected within a distance of 10 km along a continuous altitudinal gradient. Average daily mean temperatures and sums of precipitation for each week were recorded at each location during the experimental period (Fig. 1). At each location, the plants were randomly placed in three replications in one row oriented in a North to South direction, with 30 cm distance between plants. Each pot was supplied with 22 g of Osmocote™, controlled-release fertilizer with release rate 3–4 months (Scotts UK Ltd., Nottingham, UK) containing 14.0 % (w/w) N, 4.2 % (w/w) P, 11.6 % (w/w) K plus micronutrients. The plants were watered as needed according to daily rainfall by a tempo-

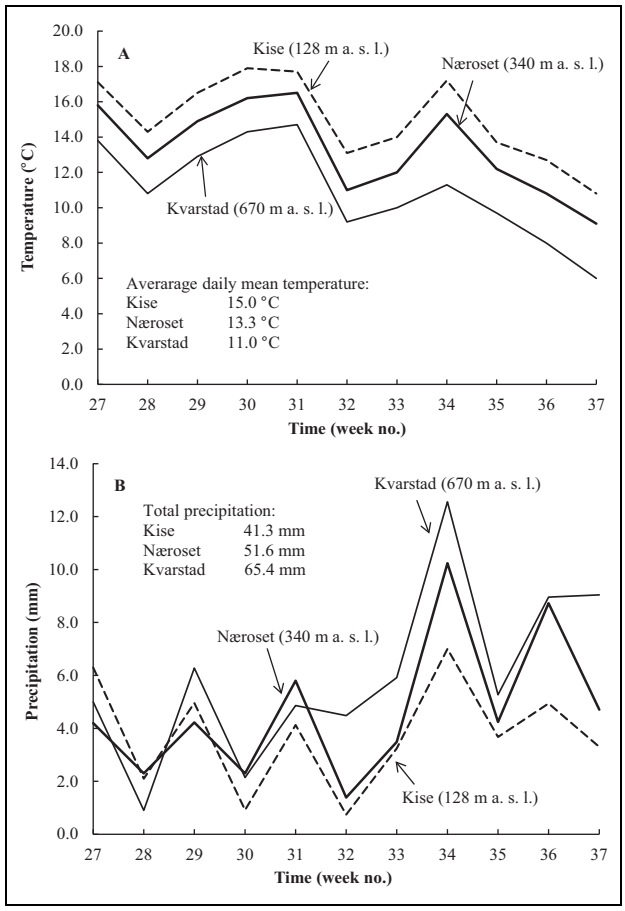


Fig. 1. Average daily mean temperature (A) and total precipitation (B) at the three altitudinal locations for each week during the experimental period (year 2011).

rary installed drip irrigation system. Fully ripe berries from the three plants in each replication at each growing site were harvested twice a week and combined to one sample before freezing. Visual care was taken to ensure that fruits of equal maturity were picked at each harvest at all locations. The number and weight of fruits from each plant and harvest time were recorded throughout the harvest period (weeks 29 to 37). The fruits were frozen within two hours after harvest and stored at −20 °C until analysis. Fruits harvested in weeks 30–34 were used for the chemical analyses. In order to avoid the confounding dilution effect of fruit size on the concentration of fruit chemical constituents (Remberg et al. 2010), analysis data were expressed on dry weight basis only.

Chemical analyses

pH, soluble solids (SS), titratable acids (TA), dry matter (DM) and L-ascorbic acid (L-AA). Raspberry fruits (150 g) were thawed over-night at room temperature, hand-pressed and filtered (Whatman, 113V type, Buckinghamshire, Germany) to obtain juice for analyses of pH, SS and TA, which were performed as described by Remberg et al. (2010) and expressed as g (10 g of DW)$^{-1}$. Dry matter content was determined by drying homogenized fruits (6 g) in an oven (Termaks, Type TS 8136, Bergen, Norway) for 24 hours at 100 °C. DM was expressed as % [g (100 g of fresh weight (FW))$^{-1}$].

L-AA was quantified in accordance with the method previously described by Williams et al. (1973) and Wold et al. (2004). L-AA analyses were conducted on an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a Zorbax SB-C18 column (4.6 × 250 mm, 5 μm) (Agilent Technologies, USA). L-AA was detected at 254 nm and quantified by external standard. L-AA content was expressed as mg (10 g DW)$^{-1}$.

Ferric reducing antioxidant power (FRAP), total monomeric anthocyanins (TMA) and total phenolics (TP). For analyses of antioxidant capacity (AOC) by the FRAP assay, TMA and TP, frozen berries were homogenized (MR400, Braun, Karlsruhe, Germany). Samples (3 g) were extracted with 1 mM HCl (37 %) in methanol (30 ml) as described by Remberg et al. (2010). The extracts were stored at −20 °C until analysis. FRAP, TMA and TP were analysed using an automatic photometric equipment, KoneLab 30i (Thermo Electron Corp., Vantaa, Finland).

The FRAP assay was performed as described by Remberg et al. (2010), and the antioxidant capacity was calculated as μmol (10 g DW)$^{-1}$. TMA were determined by the pH-differential method as previously described by Giusti and Wrolstad (2001). TMA was quantified as cyanidin-3-O-glucoside equivalents (CGE) mg (10 g DW)$^{-1}$. TP was determined by the Folin-Ciocalteu method as described previously by Slinkard and Singelton (1977) and Nwankno et al. (2012). The TP was calculated as gallic acid equivalents (mg (10 g DW)$^{-1}$).

Extraction and quantification of individual sugars and organic acids. Freeze-dried material was weighted (60 ± 2 mg) into a 2 mL Eppendorf vial and extracted at room temperature for 30 min as described by Mazur et al. (2014).

Organic acids (citrate and malate) were quantified following extract dilution (1:20) by anion exchange HPLC on a Dionex IonPac AS11-HC 4 × 250 mm column [Dionex (UK) Ltd. Camberley, UK] fitted with a 4 × 50 mm guard column according to the method described by Mazur et al. (2014).

Sugars were quantified following extract dilution (1:500) by anion exchange chromatography on a Dionex Carbopac PA-100 250 × 4 mm column [Dionex (UK) Ltd. Camberley, UK] according to the method described by Mazur et al. (2014).

Extraction and quantification of individual polyphenols. For extraction and quantification of individual polyphenols 100 ± 2 mg of freeze dried berry powder was weighted into 15 ml amber glass vials sealed with a screw cap containing a PTFE liner (SULPELCO, Sigma-Aldrich, UK) and extracted as described by Mazur et al. (2014).

The chemical analysis of the fruit extracts was performed on a high performance liquid chromatography (HPLC) system consisting of a quaternary pump (Agilent 1260), a DAD (Agilent 1260), a column temperature control device (Agilent 1260) and an autosampler Thermostat (Agilent 1290) coupled to a Triple Quadrupole Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA) with the instrument settings described in Mazur et al. (2014). Individual polyphenols were quantified by reference to an external calibration curve generated using purchased standards. The following polyphenolic standards for raspberry extracts were purchased from Extrasynthese Ltd. (Genay, France): cyanidin-3-O-glucoside chloride, cyandidin-3-O-rutinoside chloride, cyanidin-3-O-sambubioside chloride, cyanidin-3-O-sophoroside chloride, cyanin chloride, kaempferol glucoside, quercetin-3-O-glucuronide and hyperoside. Morin and ellagic acid were purchased from Sigma-Aldrich (Dorset, UK).

Statistical analyses

Statistical analyses of all data were performed using GenStat for Windows, 15th Edition [15.2.0.0821 (64-bit edition) VSN International Ltd., Hemel Hempstead, UK]. A two-way analysis of variance (ANOVA) was performed on each individual metabolite or other fruit quality parameter. Significant ($P \leq 0.05$) differences between means were estimated, followed by a multi-comparison test (Fisher's protected LSD). Significance of the main effects of location and week of harvest and their two factor interaction was assessed. To examine the relationships between measured parameters a Pearson correlation analysis was used.

Results

Fruit maturation was delayed and total fruit yield was lowered with increasing altitude, in parallel with decreasing growth temperature and increasing precipitation at the three growth locations (Figs. 1, 2; Table 1). While all plants started with the same yield potential, their realised yield potential decreased with increasing altitude of the locations. This was mainly due to delayed maturation resulting in an increasing proportion of berries that did not reach maturity at the higher altitudes. The fresh weight of fruits increased with altitude in parallel with declining temperature, while it decreased at the end of the harvest season at all locations (Table 1).

Averaged over the five harvest weeks, only fruit DW and the concentrations of L-AA, TP and FRAP were significantly affected by the growth environments established along the altitudinal gradient, whereas SS, TA, L-AA, and TMA were highly significantly affected by week of harvest (Table 2). While the concentration of L-AA generally increased with progress of harvest, SS, TA, and TMA showed the opposite trend and decreased during the harvest period. A substantial increase in TMA in week 31 at Næroset, and a respective decrease in TMA in week 33 at Kvarstad, coincided with periods of relatively high, respective low temperatures. Neither TP nor FRAP concentrations changed significantly during the harvest period.

The major sugars detected in the fruit by targeted analyses were fructose, glucose and sucrose, and the main organic acids were citrate and malate (Table 3). The mean concentrations of glucose and malate across all harvest weeks increased significantly, while sucrose decreased with increasing altitude. Fructose and citrate were the most abundant sugar and organic acid, respectively, in raspberry fruits, and, as means across all harvest weeks, neither of them varied significantly between locations. The highest concentration of ellagic acid was detected in fruit harvested at Næroset, and lowest at Kise, while the contents did not vary significantly between locations for hyperoside and quercetin glucoside (Querc-

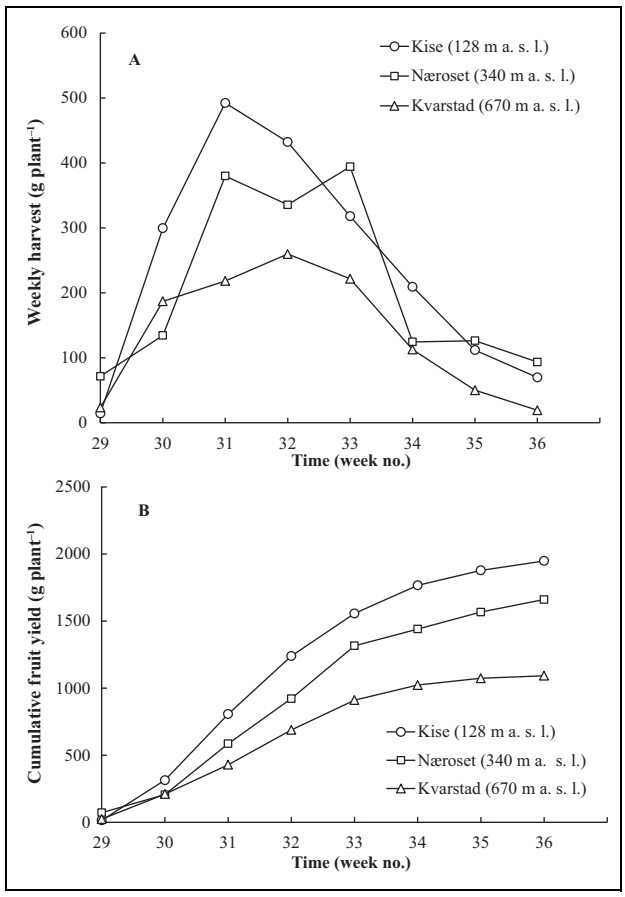


Fig. 2. Weekly (A) and cumulative (B) fruit harvests of 'Glen Ample' raspberry plants grown at the three altitudinal locations for each week during the experimental period.

Table 1. Effects of altitudinal location on fruit maturation, total fruit yield, fruit weight, number of harvested and not harvested fruits of 'Glen Ample' red raspberry.

Location (m a. s. l.)	Days to 50 % harvest[a]	Total fruit yield (g plant^{-1})[b]	Fruit weight (g)[c]	No. of harvested fruits (g plant^{-1})[b]	Total no. of harvested and not harvested fruits[b]
Kise (128)	33.3 a[d]	1977.1 a	5.7 b	345.7 a	383.9 a
Næroset (340)	36.0 ab	1679.3 a	5.7 b	297.4 a	323.4 a
Kvarstad (670)	38.7 b	1092.6 b	6.5 a	165.4 b	325.0 a
Mean	36.0	1583.0	6.0	269.5	344.1
P – value	≤ 0.010	≤ 0.001	≤ 0.001	≤ 0.001	NS

[a] Counted from date of placement of plants at the locations (July 4). [b] The sum for the harvest period (weeks 29–37). [c] Average for the harvest period (weeks 29–37). [d] Mean values (n = 9) in columns with different letters are significantly different according to Fisher's comparison test at P = 0.05. NS: not significant.

Table 2. Effects of altitudinal location and week of harvest on red raspberry fruit dry weight, soluble solids (SS), titratable acids (TA), SS/TA ratio, L-ascorbic acid (L-AA), total monomeric anthocyanins (TMA), total phenolics (TP), and antioxidant activity (FRAP).

Location/ Week of harvest	Fruit weight (g DW)	SS (%)	TA (%)	SS/TA ratio	L-AA (mg (10 g DW)$^{-1}$)	TMA (mg (10 g DW)$^{-1}$)	TP (mg (10 g DW)$^{-1}$)	FRAP (µmol (10 g DW)$^{-1}$)
Kise (128 m a. s. l.)								
30	0.9	8.5	1.9	4.5	22.1	27.6	202.3	29.1
31	1.0	8.5	1.5	5.7	20.8	27.4	206.0	28.5
32	0.8	8.4	1.7	4.9	24.7	25.5	206.6	29.5
33	0.6	8.4	1.6	5.3	25.2	23.8	203.8	28.5
34	0.5	8.2	1.5	5.5	25.7	22.6	204.2	29.8
Mean	0.8 a[a]	8.4 a	1.7 a	5.2 a	23.7 a	25.4 a	204.6 a	29.1 ab
Næroset (340 m a. s. l.)								
30	0.9	8.4	2.0	4.2	21.5	23.4	192.3	27.0
31	0.9	8.6	1.8	4.8	22.1	33.1	211.5	30.6
32	0.8	8.4	1.8	4.7	21.4	24.6	201.0	29.3
33	0.6	8.2	1.4	5.9	22.5	22.6	205.4	31.5
34	0.5	7.8	1.3	6.0	22.6	20.3	190.4	28.0
Mean	0.7 b	8.3 a	1.7 a	5.1 a	22.0 b	24.2 a	200.1 a	29.3 a
Kvarstad (670 m a. s. l.)								
30	0.9	8.3	1.8	4.6	23.8	27.9	183.2	27.7
31	0.9	8.5	1.7	5.0	21.4	28.4	191.9	28.4
32	0.9	8.1	1.8	4.5	23.3	26.9	179.8	26.4
33	0.8	8.3	1.4	5.9	23.9	21.7	187.1	28.2
34	0.6	7.6	1.3	5.8	22.4	23.2	184.9	27.0
Mean	0.8 a	8.2 a	1.6 a	5.2 a	23.0 ab	25.6 a	185.4 b	27.5 b
Source of variation (ANOVA)								
Location (A)	***	NS	NS	NS	**	NS	***	*
Harvest week (B)	***	***	***	***	**	***	NS	NS
A × B	***	NS	***	***	*	NS	NS	NS

[a] Mean values (n = 9) in columns with different letters are significantly different according to Fisher's comparison test at P = 0.05. Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. NS: not significant.

Gluc) (Table 3). Among the 9 quantified anthocyanins, cyanidin sophoroside (Cyan-Soph) was by far the most prevalent one, followed by cyanidin-3-O-glucoside (Cyan-Gluc) and cyanidin-3-O-rutinoside (Cyan-Rut) (Table 4). The average levels of Cyan-Soph, cyanidin-3-O-sambubioside (Cyan-Samb), and pelargonidin sophoroside (Pel-Soph) rose, while Cyan-Rut dropped significantly with increasing altitude. The concentrations of the other anthocyanins did not vary significantly among the altitudinal locations (Table 4). The interactions between location and week of harvest were insignificant for all sugars, organic acids, flavonols and anthocyanins except for sucrose which varied during the harvest period to a larger extent at Kise than at Næroset and Kvarstad (Tables 3 and 4).

Pearson's correlation analysis revealed significant correlations between several quality parameters (data not presented). Fruit dry weight was positively correlated with SS ($R = 0.66$, $P < 0.01$), TA ($R = 0.67$, $P < 0.01$), TMA ($R = 0.76$, $P < 0.001$), and sum of acids ($R = 0.74$, $P < 0.01$), but negatively correlated with L-AA ($R = -0.53$, $P < 0.05$). FRAP was highly positively correlated with TP ($R = 0.82$, $P < 0.001$), while no correlation was present with L-AA or TMA. Highly significant positive intercorrelations were also detected between the phenolic compounds hyperoside and Querc-Gluc (data not presented).

When values for the various fruit constituents at any time of harvest, and across the three locations, were correlated with temperature and precipitation conditions during the actual week of harvest and the preceding one

Table 3. Effects of altitude and week of harvest on phytochemical composition of 'Glen Ample' red raspberry fruit.[a]

Location/ Week of harvest	Fructose	Glucose	Sucrose	Citrate	Malate	Hyperoside	Ellagic Acid	Querc-Gluc
Kise (128 m a. s. l.)								
30	192.5	167.3	99.2	175.7	5.52	0.0482	0.0284	0.0409
31	187.8	167.4	120.5	137.0	1.59	0.0334	0.0364	0.0214
32	205.8	175.2	136.0	149.4	3.26	0.0285	0.0516	0.0206
33	214.2	185.5	120.4	136.5	4.27	0.0342	0.0423	0.0244
34	192.7	171.4	99.7	122.6	3.62	0.0311	0.0414	0.0196
Mean	198.6 a[b]	173.3 b	115.2 a	144.2 a	3.65 c	0.0351 a	0.0400 b	0.0254 a
Næroset (340 m a. s. l.)								
30	191.8	166.1	51.2	145.9	6.5	0.0481	0.0761	0.0423
31	197.9	178.5	63.7	139.5	3.17	0.0395	0.1117	0.0297
32	210.4	186.8	106.5	140.6	5.63	0.0301	0.0367	0.0221
33	227.9	207.1	129.3	134.4	4.72	0.0393	0.0625	0.0285
34	221.0	201.5	96.0	121.0	3.76	0.0355	0.1993	0.0220
Mean	209.8 a	188.0 a	89.4 b	136.3 a	4.76 b	0.0385 a	0.0973 a	0.0290 a
Kvarstad (670 m a. s. l.)								
30	201.6	183.2	98.1	150.2	7.19	0.0477	0.0662	0.0450
31	217.6	196.8	87.6	153.6	6.27	0.0457	0.0578	0.0319
32	204.3	185.4	91.1	160.7	6.07	0.0338	0.0369	0.0244
33	215.6	202.5	87.8	139.2	5.96	0.0374	0.0447	0.0235
34	206.7	197.1	79.9	124.4	5.79	0.0325	0.0920	0.0165
Mean	209.1 a	193.0 a	88.9 b	145.6 a	6.25 a	0.0394 a	0.0595 ab	0.0283 a
Source of variation (ANOVA)								
Location (A)	NS	***	***	NS	***	NS	*	NS
Harvest week (B)	*	***	**	***	**	***	NS	***
A × B	NS	NS	**	NS	NS	NS	NS	NS

[a] Expressed as μg mg^{-1} fruit DW. [b] Mean values (n = 9) in columns with different letters are significantly different according to Fisher's comparison test at P = 0.05. Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. NS: not significant.

to three weeks, highly interesting climatic effects were revealed (Table 5, Fig. 3). First of all, temperature and precipitation during the various harvest weeks were usually negatively correlated, and therefore, their effects on the various fruit constituents were usually also inversely related. This correlation analysis also revealed that it was the climatic conditions during the harvest week and the one immediately preceding harvest that mainly influenced fruit chemical composition. Temperature during these two weeks was positively correlated with fruit weight, SS, TA, TMA, and TP, while precipitation during the same period had the opposite effect. SS/TA ratio, and L-AA were negatively correlated with temperature, and positive correlated with precipitation, during the harvest week, and the week before harvest. Fruit yield on the other hand, was affected over an extended period of time, the positive effect of temperature being particularly marked in the period two weeks before harvest. Generally, precipitation tended to have the strongest impact during the harvest week and temperature during the week preceding harvest. Correlations with the climatic conditions prevailing two and three weeks before harvest gradually became insignificant, and in some cases, the effects were even reversed (Table 5).

Similar climatic relations were found for the targeted analysis data (Fig. 3). The levels of glucose and fructose were negatively correlated with temperature and positively correlated with precipitation during the week of harvest. Sucrose on the other hand, was not significantly correlated with these climatic factors during the harvest week, but was positively correlated with temperature one and two weeks before harvest, and, to a lesser degree, negatively correlated with precipitation during the week immediately preceding harvest. Concentration of malate was negatively correlated with temperature and positively correlated with precipitation in the current week and the

Table 4. Effects of altitude and week of harvest on anthocyanin composition of 'Glen Ample' raspberry fruit.[a]

Location/ Week of harvest	Cyanin	Cyan-Soph	Cyan-Samb	Cyan-Rut	Cyan-Gluc	Cyan-Soph-Rham[b]	Pel-Soph[b]	Cyan-Xyl-Rut[b]	Pel-Gluc-Rut[b]
Kise (128 m a. s. l.)									
30	0.04327	2.029	0.04115	0.381	0.496	0.0590	0.0261	0.01459	0.00864
31	0.04723	1.906	0.03934	0.612	0.591	0.0783	0.0293	0.01894	0.01324
32	0.03029	1.778	0.03374	0.280	0.372	0.0535	0.0287	0.01263	0.00942
33	0.02932	1.744	0.03238	0.212	0.342	0.0378	0.0233	0.00915	0.00569
34	0.03303	2.155	0.04099	0.267	0.414	0.0454	0.0325	0.01136	0.00812
Mean	0.03663 a[c]	1.923 b	0.03752 b	0.351 a	0.443 a	0.0548 a	0.0280 b	0.01333 a	0.00902 a
Næroset (340 m a. S. L.)									
30	0.03138	2.090	0.04125	0.279	0.414	0.0494	0.0299	0.01279	0.00796
31	0.04862	2.242	0.04601	0.498	0.576	0.0692	0.0325	0.01678	0.0110
32	0.03222	1.986	0.03616	0.308	0.421	0.0535	0.0281	0.01193	0.00789
33	0.02758	2.120	0.03768	0.263	0.440	0.0399	0.0323	0.00921	0.00693
34	0.03230	2.234	0.03993	0.287	0.455	0.0453	0.0305	0.01087	0.00689
Mean	0.03442 a	2.134 b	0.04021 ab	0.327 ab	0.461 a	0.0514 a	0.0307 b	0.01231 a	0.00813 a
Kvarstad (670 m a. S. L.)									
30	0.03435	2.295	0.04291	0.263	0.409	0.0512	0.0361	0.01275	0.00933
31	0.05702	2.479	0.04783	0.389	0.549	0.0594	0.0288	0.01484	0.00783
32	0.03204	2.517	0.04385	0.219	0.414	0.0468	0.0379	0.01100	0.00784
33	0.02123	2.303	0.04097	0.224	0.433	0.0338	0.0395	0.00786	0.00700
34	0.02783	2.620	0.04674	0.269	0.492	0.0463	0.0458	0.01041	0.00896
Mean	0.03449 a	2.443 a	0.04446 a	0.273 b	0.459 a	0.0475 a	0.0376 a	0.01137 a	0.00819 a
Source of variation (ANOVA)									
Location (A)	NS	***	*	*	NS	NS	*	NS	NS
Harvest week (B)	***	NS	NS	***	***	***	NS	***	NS
A × B	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Expressed as μg mg^{-1} DW fruit. [b] Calculated as cyanidin-3-O-sambubioside equivalent. [c] Mean values (n = 9) in columns with different letters are significantly different according to Fisher's comparison test at P = 0.05. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. NS: not significant.

week preceding harvest, while citrate showed no consistent relationship with these climatic factors. None of these correlations were significant (Fig. 3). Of the 9 anthocyanins detected, the majority (6 species) were strongly positively correlated with temperature and negatively correlated with precipitation during the week of harvest and, to a lesser extent, the week preceding harvest. There was also a consistent tendency that the positive effect of temperature current with and immediately preceding harvest changed to become negative during the periods two and three weeks before harvest. The remaining three anthocyanins, Cyan-Samb, Cyan-Soph, and Pel-Soph showed the opposite relationship and were negatively correlated with temperature and positively correlated with precipitation, particularly during the week preceding harvest and, to a lesser extent, also with the conditions current with and two weeks preceding harvest. The other polyphenolic compounds analysed did not show any consistent relationship with temperature and precipitation, although there was a tendency that ellagic acid and hyperoside were negatively affected by warm temperature conditions during the last two weeks before fruit harvest (Fig. 3). Generally, daily maximum, mean, and minimum temperatures affected the various fruit constituents in the same way, but not to the same degree. Usually, maximum temperatures conferred the strongest positive correlations and minimum temperatures the strongest negative correlations.

Discussion

Although significant effects of environmental conditions on chemical composition on both fruit and other plant

Table 5. Pearson correlations between weekly mean temperature (°C) and weekly sum of precipitation (mm) with selected compounds[a] and quality attributes of 'Glen Ample' red raspberry fruit.

	Harvest week		1 week before harvest		2 weeks before harvest		3 weeks before harvest	
	Temp.	Precip.	Temp.	Precip.	Temp.	Precip.	Temp.	Precip.
Precipitation	−0.64**[b]		−0.60*		−0.04		−0.35	
Yield	0.39	−0.37	0.64*	−0.60**	0.75***	0.06	0.04	−0.32
Fruit weight	0.46	−0.60**	0.53*	−0.44*	−0.09	0.09	−0.49*	0.04
SS	0.67**	−0.49*	0.64**	−0.55**	0.38	−0.14	−0.08	−0.20
TA	0.46	−0.61**	0.53*	−0.33	−0.06	0.11	−0.13	−0.12
SS/TA	−0.35	0.60**	−0.48*	0.22	0.15	−0.16	0.14	0.09
L-AA	−0.31	0.26	−0.17	0.11	0.26	−0.17	0.44	0.24
TMA	0.58*	−0.78***	0.52*	0.04	−0.26	−0.11	−0.51**	0.06
TP	0.59*	−0.21	0.50*	−0.27	0.63*	−0.41	0.48	−0.63**
FRAP	0.28	0.05	0.15	−0.16	0.45	−0.28	0.31	−0.37

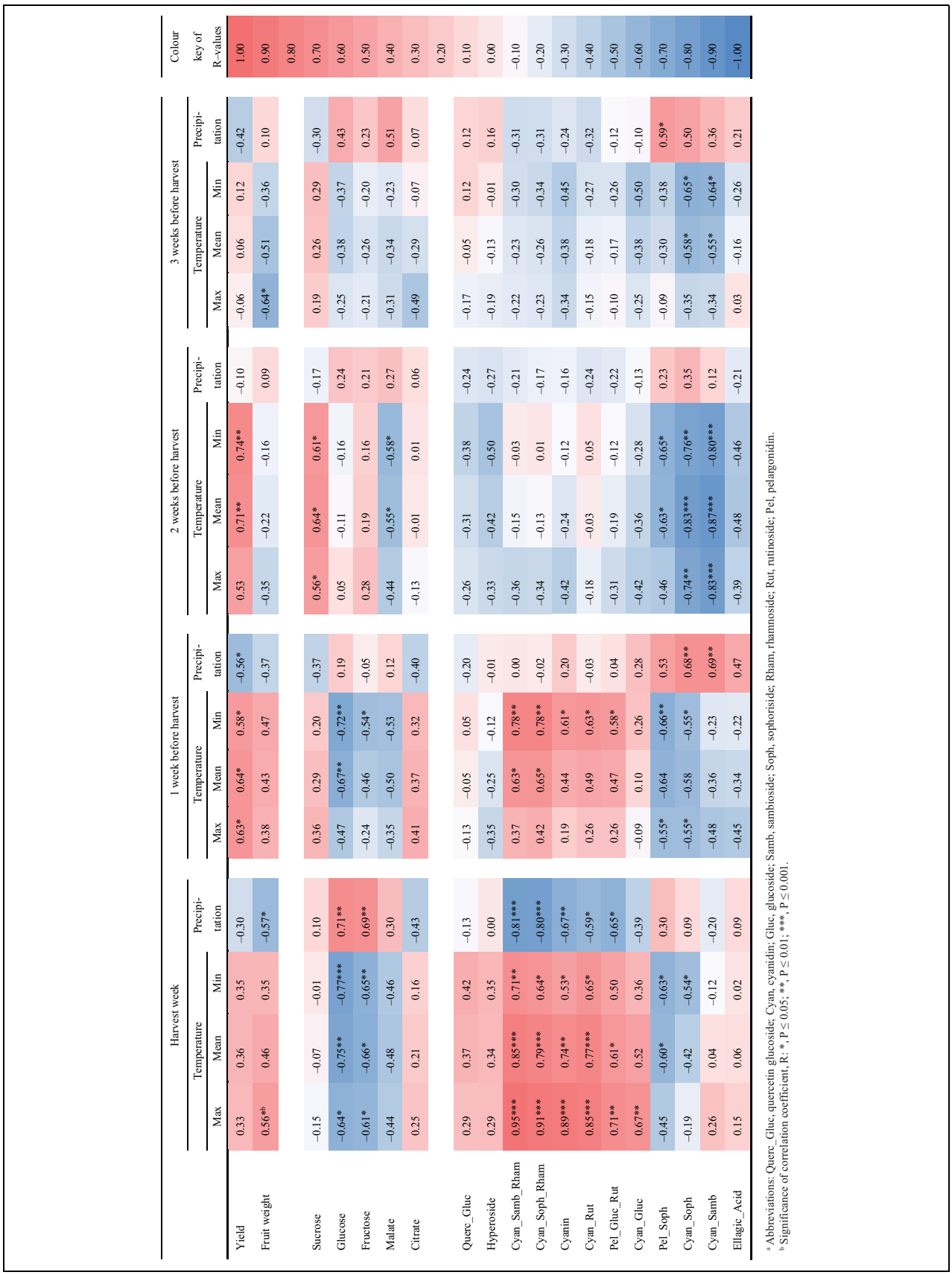
[a] Abbreviations: SS, soluble solids; TA, titratable acids, L-ascorbic acid (L-AA), total monomeric anthocyanins (TMA), total phenolics (TP), and antioxidant activity (FRAP). [b] Significance of correlation coefficient, R: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

tissues are well documented (Given 1985; Moore et al. 2005; Jaakola and Hohtola 2010; Remberg et al. 2010; Mazur et al. 2014), environmental factors such as temperature, photoperiod, radiation, and light quality may have dissimilar or even opposing effects on the concentrations of the various chemical constituents of raspberry fruit (Given 1985). Furthermore, in experiments like the present one, changing temperature and precipitation conditions during the harvest period will even out and mask the average effects of these climatic factors for the total harvest period. These inherent analytical problems could be circumvented by correlating fruit chemical composition for the various harvests and across all locations with temperature and precipitation data during the actual week of harvest and the preceding weeks (Table 5, Fig. 3). Such correlation analyses, which identified the immediate effects of the current environment as well as the after-effects of the preceding conditions, revealed marked and consistent effects of both temperature and precipitation on raspberry fruit chemical composition and allowed the separation of the effects of temperature and precipitation.

The results of these analyses revealed that it was mainly during the final stages of fruit maturation that these environmental factors were exerting their effects on fruit chemical composition, and furthermore, that within the ranges established in this experiment, increasing temperature and precipitation had opposite effects, temperature enhancing and precipitation reducing the concentration of important fruit quality constituents such as SS, TA, TMA, and TP (Table 5). These results are consistent with the results of Wang et al. (2009) who reported that, with the exceptions of ellagic acid and the flavonols, synthesis of most quality-related fruit constituents took place during the final stages of fruit maturation. Performance of the same procedure using the targeted analysis data also facilitated some insight into the specific temporal changes in the impact of the climatic factors as well as the interrelations between the various compounds (Fig. 3). For example, while the concentration of a majority of six anthocyanin species was strongly enhanced by warm temperature and reduced by precipitation during the final week of maturation, another group of three species exhibited the opposite responses. The analysis also made it evident that, in the latter group; it was the conditions prevailing one to three weeks before maturity that had the strongest impact. A similar, but opposite relationship was observed for the monosaccharides fructose and glucose versus sucrose suggesting that interconversion of sucrose to monosaccharides was facilitated by low temperature (Fig. 3).

The concentrations of nutritional and potentially health-beneficial phytochemicals and the FRAP values, listed in Table 2, were within ranges previously reported for raspberry (Anttonen and Karjalainen 2005; Connor et al. 2005; Rao and Snyder 2010; Remberg et al. 2010; Mazur et al. 2014). Highly significant correlation between TP and FRAP and lack of significant correlations between TMA with TP, indicate that other phenolic compounds than anthocyanin were major contributors to the antioxidant capacity in raspberry fruit (data not shown, cf. Borges et al. 2009). The concentration of anthocyanins is known to vary among raspberry cultivars (Rao and Snyder 2010), and consistent with previous studies (Mullen et al. 2002; Remberg et al. 2010), 'Glen Ample' was found to have midrange concentrations.

Under controlled environment conditions, the concentration of L-AA was found to increase with increasing day length (Mazur et al. 2014) and decreasing temperature



	Harvest week				1 week before harvest				2 weeks before harvest				3 weeks before harvest			
	Temperature			Precipitation	Temperature			Precipitation	Temperature			Precipitation	Temperature			Precipitation
	Max	Mean	Min		Max	Mean	Min		Max	Mean	Min		Max	Mean	Min	
Yield	0.33	0.36	0.35	–0.30	0.63*	0.64*	0.58*	–0.56*	0.53	0.71**	0.74**	–0.10	–0.06	0.06	0.12	–0.42
Fruit weight	0.56[b]	0.46	0.35	–0.57*	0.38	0.43	0.47	–0.37	–0.35	–0.22	–0.16	0.09	–0.64*	–0.51	–0.36	0.10
Sucrose	–0.15	–0.07	–0.01	0.10	0.36	0.29	0.20	–0.37	0.56*	0.64*	0.61*	–0.17	0.19	0.26	0.29	–0.30
Glucose	–0.64*	–0.75**	–0.77***	0.71**	–0.47	–0.67**	–0.72**	0.19	0.05	–0.11	–0.16	0.24	–0.25	–0.38	–0.37	0.43
Fructose	–0.61*	–0.66*	–0.65**	0.69**	–0.24	–0.46	–0.54*	–0.05	0.28	0.19	0.16	0.21	–0.21	–0.26	–0.20	0.23
Malate	–0.44	–0.48	–0.46	0.30	–0.35	–0.50	–0.53	0.12	–0.44	–0.55*	–0.58*	0.27	–0.31	–0.34	–0.23	0.51
Citrate	0.25	0.21	0.16	–0.43	0.41	0.37	0.32	–0.40	–0.13	–0.01	0.01	0.06	–0.49	–0.29	–0.07	0.07
Querc_Gluc	0.29	0.37	0.42	–0.13	–0.13	–0.05	0.05	–0.20	–0.26	–0.31	–0.38	–0.24	–0.17	–0.05	0.12	0.12
Hyperoside	0.29	0.34	0.35	0.00	–0.35	–0.25	–0.12	–0.01	–0.33	–0.42	–0.50	–0.27	–0.19	–0.13	–0.01	0.16
Cyan_Samb_Rham	0.95***	0.85***	0.71**	–0.81***	0.37	0.63*	0.78**	0.00	–0.36	–0.15	–0.03	–0.21	–0.22	–0.23	–0.30	–0.31
Cyan_Soph_Rham	0.91***	0.79***	0.64*	–0.80***	0.42	0.65*	0.78**	–0.02	–0.34	–0.13	0.01	–0.17	–0.23	–0.26	–0.34	–0.31
Cyanin	0.89***	0.74**	0.53*	–0.67**	0.19	0.44	0.61*	0.20	–0.42	–0.24	–0.12	–0.16	–0.34	–0.38	–0.45	–0.24
Cyan_Rut	0.85***	0.77***	0.65*	–0.59*	0.26	0.49	0.63*	–0.03	–0.18	–0.03	0.05	–0.24	–0.15	–0.18	–0.27	–0.32
Pel_Gluc_Rut	0.71**	0.61*	0.50	–0.65*	0.26	0.47	0.58*	0.04	–0.31	–0.19	–0.12	–0.22	–0.10	–0.17	–0.26	–0.12
Cyan_Gluc	0.67**	0.52	0.36	–0.39	–0.09	0.10	0.26	0.28	–0.42	–0.36	–0.28	–0.13	–0.25	–0.38	–0.50	–0.10
Pel_Soph	–0.45	–0.60*	–0.63*	0.30	–0.55*	–0.64	–0.66**	0.53	–0.46	–0.63*	–0.65*	0.23	–0.09	–0.30	–0.38	0.59*
Cyan_Soph	–0.19	–0.42	–0.54*	0.09	–0.55*	–0.58	–0.55*	0.68**	–0.74**	–0.83***	–0.76**	0.35	–0.35	–0.58*	–0.65*	0.50
Cyan_Samb	0.26	0.04	–0.12	–0.20	–0.48	–0.36	–0.23	0.69**	–0.83***	–0.87***	–0.80***	0.12	–0.34	–0.55*	–0.64*	0.36
Ellagic_Acid	0.15	0.06	0.02	0.09	–0.45	–0.34	–0.22	0.47	–0.39	–0.48	–0.46	–0.21	0.03	–0.16	–0.26	0.21

[a] Abbreviations: Querc_Gluc, quercetin glucoside; Cyan, cyanidin; Gluc, glucoside; Samb, sambioside; Soph, sophoriside; Rham, rhamnoside; Rut, rutinoside; Pel, pelargonidin.
[b] Significance of correlation coefficient, R: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Fig. 3. Pearson correlations between weekly mean temperature (°C) and weekly sum of precipitation (mm) with targeted compounds[a] quantified in 'Glen Ample' red raspberry fruit.

(Remberg et al. 2010). However, while Remberg et al. (2010) observed a stepwise and highly significant decrease in L-AA content with increasing temperature from 12 to 24 °C, only an insignificant negative correlation with temperature during fruit maturation was observed in the present study (Table 5). It should be kept in mind, however, that the temperatures in this experiment were all within the lower range used by Remberg et al. (2010), and furthermore, that the concentrations of L-AA observed in the present experiment were at least as high as those found at 12 °C by Remberg et al. (2010). Apparently, a wider range of temperatures would have been required to expose the full range negative temperature response.

Precipitation during fruit maturation was found to have a general negative impact on fruit chemical composition (Table 5, Fig. 3). Since only moderate rainfalls were experienced (Fig. 1), and since watering was adjusted according to the daily rainfall, it is very unlikely that this negative effect was due to excessive water supply as such. On the other hand, rainy weather is associated with increased cloudiness which is known to reduce daily light energy, predominantly by absorption of long-wave visible radiation (Monteith and Unsworth 2013). It is therefore, most likely that the observed effects of rainfall were indirect, and caused by modified light conditions. Thus, in a review of the available literature in the field, Given (1985) conclude that "increased sunshine hours and warm dry weather are associated with raised SS content and anthocyanin concentrations in raspberries and blackberries". This is exemplified in the highly significant negative correlation of TMA with precipitation and the associated positive correlation with temperature presented in Table 5 and concurs with the findings of a recent report by Remberg et al. (2014). In a 7-year field trial in Norway with the raspberry cultivars 'Algonquin' and 'Glen Ample', SS content was highly significantly negatively correlated with July precipitation, while SS as well as FRAP were highly positively correlated with temperature and global radiation in the same period. However, in contrast to previous studies in controlled environments (Remberg et al. 2010; Mazur et al. 2014), the results in Table 5 demonstrate a consistent decrease in TMA at all locations during the harvest period. This was associated with a steady increase in precipitation during that period (Fig. 1, Table 2) and further demonstrates that decreased radiation associated with an increasing trend in precipitation can have a large effect on anthocyanin synthesis. However, consistent with the findings of Remberg et al. (2010), it was found that major anthocyanins were differently affected by temperature, some increasing (such as Cyan-Soph-Rham), and others (such as Cyan-Soph) decreasing with temperature (Fig. 3), thus evening out the over-all effect on TMA across all harvests (Table 2).

In conclusion, the present results and those by Remberg et al. (2014), demonstrate that weather conditions during fruit maturation can have significant effects on raspberry fruit chemical composition. In the cool Nordic climate, and at the lower temperature range as established in the present experiment by growing plants at different elevations, the concentration of a range of fruit quality-related parameters such as SS, TA, TMA, and TP was enhanced by increasing temperature during the final two weeks of fruit maturation, while precipitation during the same period had the opposite effect (Table 5, Fig. 3). However, under greenhouse conditions, temperatures as high as 24 °C were shown to have the opposite effect (Remberg et al. 2010). It was evident, however, that the precipitation effect was not directly related to plant water supply, but was rather, an indirect effect related to increased cloudiness and reduced radiation during rainy weather (cf. Remberg et al. 2014). However, the effects of environmental factors can vary vastly between genotypes (Connor et al. 2002; 2005), and often, selection of the right cultivar may have greater effect on fruit quality than manipulation of the environment.

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Addresses of authors: Sebastian Piotr Mazur (corresponding author), Bioforsk – Norwegian Institute for Agricultural and Environmental Research, NO-1432 Ås, Norway and Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway; A. Sønsteby and A. Nes, Bioforsk – Norwegian Institute for Agricultural and Environmental Research, NO-1432 Ås, Norway; A. B. Wold, Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway; A. Foito, S. Freitag, S. Verrall and D. Stewart, Environment and Biochemical Sciences, the James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK, and O. M. Heide, Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, NO-1432 Ås, Norway, e-mail (corresponding author): sebastian.mazur@bioforsk.no.