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ARTICLE

Influence of Postflowering Temperature on Fruit Size and Chemical Composition of Glen Ample Raspberry (*Rubus idaeus* L.)

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The effects of postflowering temperature on the fruit chemical composition of Glen Ample raspberries were studied under controlled environment conditions. The berry weight decreased significantly with increasing temperature (12, 18, and 24 °C) and with progress of the harvest period. Because the moisture content increased in parallel with the berry weight, the antioxidant capacity (AOC) and the concentration of a range of bioactive compounds decreased with decreasing temperature and progress of the harvest season when expressed on a fresh weight basis in the conventional way. Under those circumstances, dry weight units are therefore preferable. However, despite the dilution effect of large berries, the concentration of ascorbic acid (vitamin C) increased with decreasing temperature, even on a fresh weight basis. Berry AOC was closely correlated with total phenolic concentration (r = 0.958), predominantly anthocyanins and ellagitannins. While a total of 10 anthocyanins were detected, cyanidin-3-sophoroside and cyanidin-3-(2^{G}-glucosylrutinoside)-rutinoside accounted for 73% of the total, the former decreasing and the latter increasing with increasing growth temperature. By far, the most prevalent ellagitannins were lambertianin C and sanguiin H-6, both of which increased significantly with increasing temperature. It is concluded that the growth temperature has significant and contrasting effects on the concentration of a range of potentially bioactive compounds in raspberry.

KEYWORDS: Antioxidant capacity; anthocyanins; climate; ellagic acid; ellagitannins; raspberries; *Rubus idaeus*; temperature; total phenolics; vitamin C

INTRODUCTION

Fruits and in particular berries contain a range of bioactive constituents that are considered of essential value in human diets (*1* and references therein). A number of studies have shown that increased consumption of fruits and vegetables can have risk reduction effects against major health problems such as cancer (*2*) and cardiovascular diseases (*3*, *4*). Traditionally, the water-soluble ascorbic acid (AA, vitamin C) has played a predominant role in such considerations because of its antiscorbutic effect, but lately, dietary antioxidants have received increasing attention because of their important function in mitigating the damaging effects of oxidative stress on cells and tissues (*1*, *5*, *6*). Although a number of compounds contribute to the antioxidant capacity (AOC) of commonly consumed fruits, the total activity is mainly reflected in their total phenolic (TP) and AA concentrations (*7*, *8*). Fruits and berries are a rich source of phenolic compounds, including flavonoids, phenolic acids, and tannins, leading to an extensive number of different compounds (*9*). One of these is ellagic acid, which has recently been the focus of interests because of its demonstrated in vitro (*11*, *12*) and in vivo (*10*) anticarcinogenic, antimutagenic, and antioxidative activities. Ellagic acid is not naturally present in the free form but is conjugated with a sugar or, more commonly, as part of the ellagitannins (*13*).

Because of the potential health benefits of dietary antioxidants, much effort has been invested in analytical screening of the AOC of dietary plants, including the commonly cultivated temperate small fruits (*14*, *15*). Research in this area has mainly focused on variation among species and cultivars (*16*−*18*) and effects of postharvest handling and storage (*7*, *19*, *20*). On the other hand, little is known about the impact of environmental factors such as temperature and light conditions on fruit antioxidants and other beneficial dietary constituents.

Red raspberry (*Rubus idaeus* L.) is an economically important small fruit species and a rich source of antioxidants and phytochemicals with potential health benefits (*1*, *21*). The most abundant phenolic compounds in raspberries are anthocyanins and ellagic acid derivatives, that is, ellagitannins and ellagic acid glycosides (*18*, *22*, *23*). These compounds are the main contributors to the high AOC of raspberry (*19*, *22*−*25*), while vitamin C

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accounts for only 6% of the total AOC (*7*). Ellagic acid-containing compounds are only found in some fruits, and raspberries, together with strawberries, are major dietary sources of these constituents (*13*, *26*). In red raspberry, they constitute a major part of the TP compounds (*16*). Both the quantity and the relative proportion of these potentially bioactive compounds are shown to depend on genetic as well as environmental factors (*27*, *28*), but hardly anything is known about the specific impact of the various environmental factors. In strawberry, it was shown that the total AOC was lower in fruits grown at 18/12 °C day/night temperatures than at 30/22 °C (*29*), while the opposite trend was reported for vitamin C concentration (*30*). In raspberry, the potential effects of temperature on fruit chemical compositions are accentuated by the advent of protected cultivation for out of season production (*31*, *32*). This involves changes in the climatic environment that might influence the chemical composition of the fruits, and furthermore, the controlled environment may also be utilized to enhance fruit AOC (*33*). Therefore, we have studied the influence of growth temperature on raspberry fruit quality. Fruiting plants were grown during fruit development and maturation in three daylight phytotron compartments with different constant temperatures, and the effect on important fruit quality attributes was determined. Comparisons were also made with berries produced under conventional conditions in an open polyethylene tunnel.

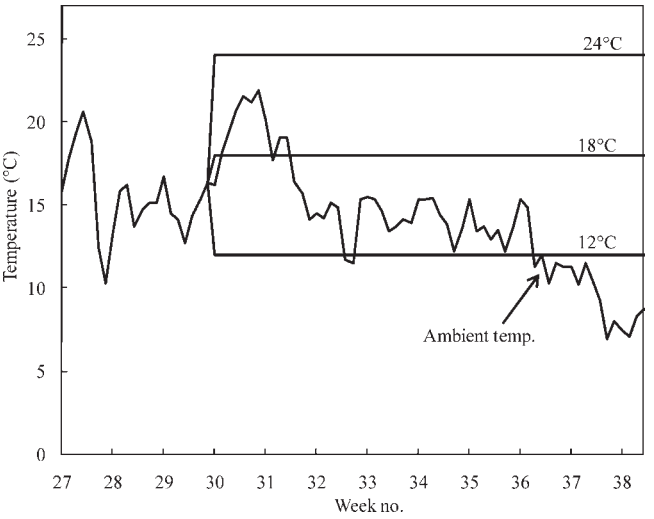

Figure 1. Temperature conditions in the three phytotron compartments and in the Haygrove polyethylene tunnel (ambient temperature) during the 8 weeks of experimentation. All plants were grown in the open Haygrove tunnel from June 1 until July 20 (week no. 30) when plants were moved into the phytotron. The ambient temperature course represents daily mean temperatures based on daily maximum and minimum temperature recordings.

MATERIALS AND METHODS

Chemicals. Oxalic acid dihydrate, acetonitrile, methanol, $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, sodium acetate, acetic acid, formic acid, potassium chloride, and sodium carbonate were obtained from Merck KGaA (Darmstadt, Germany). 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Fluka Chemie GmbH (Buchs, Switzerland). L-(+)-AA and technical methanol were obtained from BDH Prolabo (VWR International, Norway). Cyanidin-3-sophoroside was purchased from Polyphenols Laboratories AS (Sandnes, Norway). Gallic acid (3,4,5-trihydroxybenzoic acid), ellagic acid, quercetin-3-rhamnosylglucoside (rutin), and Folin−Ciocalteu's phenol reagent (2.0 N) were purchased from Sigma Chemical Co. (St. Louis, MO), and hydrochloric acid was obtained from Riedel-de Haën AG (Seelze, Germany). All solvents were of high-performance liquid chromatography (HPLC) grade, and water was of Milli-Q quality (Millipore Corp., Bedford, MA).

Plant Material and Cultivation. Pot-grown long cane raspberry plants (*R. idaeus* L. cv. Glen Ample) with a yield potential of 2.5 kg were produced as described by Sønsteby et al. (*32*). After cold storage at −2 °C over the winter, the plants were moved into an open Haygrove polyethylene tunnel on June 1, 2009, and grown for fruit production as described (*32*). All plants remained in the tunnel until July 20 (week 30) when three groups of plants were moved into daylight phytotron compartments (Ås, Norway, 59° 40′N) with temperatures of 12, 18, and 24 °C (±1 °C) and natural daylength (18−12 h), while a fourth group of plants remained in the plastic tunnel throughout the summer as shown in **Figure 1**. In the following, this treatment is referred to as ambient temperature. In the phytotron, a water vapor saturation deficit of 530 Pa was maintained at all temperatures. The developmental stage of the plants at transfer to the phytotron is shown in **Figure 2**. Each treatment comprised nine plants distributed on three randomized blocks with three plants each.

In all treatments, berries were harvested two times weekly from week 32 to 38, and the number and weight of berries were recorded. Berries harvested on weeks 33, 35, and 37 (harvest weeks 2, 4, and 6, respectively) were sampled for chemical analyses. By visual assessment, care was taken to ensure that berries of equal maturity were sampled. The samples (minimum of 25 fruits) were frozen and stored at −20 °C until processed.

Soluble Solids (SS), pH, Titratable Acidity (TA), Color, and Dry Matter (DM). The berries were thawed overnight at room temperature prior to analyses. Berry juice was obtained by squeezing the berry samples by hand and then filtered (Whatman 125 mm, Schleicher & Schuell, Germany). The juice was used for SS (Atago Palette PR-100, Japan), pH (Methrom 691 pH Meter, Herisau, Switzerland), TA (Methrom 716 DMS Titrino and 730 Sample Changer), and color analyses. Prior to the color analyses, the juice was diluted to a 5% solution with distilled water. The juice color was determined as optical density (OD) at 515 nm in a spectrophotometer (Shimadzu UV mini 1240, Japan). For the DM content, 6−7 g of homogenized berry mass was dried at +100 °C for 24 h in a drying oven followed by stabilization in a desiccator.

AA. Frozen raspberries (25 g, 6−12 fruits depending on temperature and harvest time) were added up to 150 g with 1% (w/v) oxalic acid. The material was homogenized for 1 min, filtered (B 1/2, folded, Schleicher & Schuell), and semipurified using a Sep-Pak C18 cartridge (Waters Corp., United States). The samples were then filtered through a 0.45 μm Millex HA filter (Millipore, MolsHeim, France) before HPLC analysis.

HPLC analyses was performed according to Williams et al. (*34*), 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. HPLC operation was performed by means of Chemstation software (Agilent, Germany). Separation was achieved using a 4.6 mm × 250 mm long Zorbax SB-C18 5 Micron column (Agilent Technologies, United States). The injection volume (5 μL) and isocratic elution were performed with 0.05 M KH_2PO_4 at 1 mL min^{-1} and 25 °C. AA was measured at 254 nm, and the identity of the peak and quantification of AA was performed by an authentic standard (AA in 1% oxalic acid) and by using calibration curves of freshly prepared standard solutions, respectively.

Ferric Reducing Activity Power (FRAP), Total Monomeric Anthocyanins (TMA), and TP. Slightly thawed berries were homogenized with a blender (Braun MR400, Karlsruhe, Germany). The homogenized berry mass (3 g) 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., NY), 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 analyzed. Prior to analysis, the samples were poured into a 2 mL Sarstedt micro tube (Nürnbrecht, Germany) and centrifuged at 13200 rpm for 2 min at 4 °C (Eppendorf 5415 R, Germany). The raspberry samples were extracted in triplicate.

For analyses of FRAP, TMA, and TP, KoneLab 30i (Thermo Electron Corp., Vantaa, Finland), a clinical chemical analyzer was used. The FRAP assay was carried out as described by Benzie and Strain (*35*), with some modifications as previously reported (*36*). TMA was determined by the pH differential method based on the spectral characteristics of anthocyanins (*37*), and TP was determined using the Folin−Ciocalteu method as

Figure 2. Appearance of the experimental plants at the stage of transfer into the phytotron compartments.

described in Kähkönen et al. (*38*), with modifications described in Volden et al. (*36*). Results are reported as mmol Fe^{2+} per 100 g of fresh weight (FRAP), mg cyanidin-3-glucoside equivalents (CGE) per 100 g of fresh weight (TMA), and mg gallic acid equivalents (GAE) per 100 g of fresh weight (TP).

Phenolic Compounds Analyzed by HPLC−Diode Array Detection−Mass Spectrometry (DAD-MS). Frozen raspberries were thawed for 1 min and homogenized with a hand-blender (Braun MR400, Karlsruhe, Germany). Samples (5 g) were mixed with methanol (10 mL) and homogenized with a polytron homogenizer (Kinematica CH-6010, Kriens LU Polytron, Switzerland). The samples were sonicated (Bandelin SONOREX RK 100, Bandelin Electronic GmbH & Co.) before centrifugation (4000 rpm, Eppendorf Centrifuge, 5810 R, Hamburg, Germany), both procedures for 10 min at 4 °C. After centrifugation, the supernatant was collected, and the insoluble plant material was re-extracted with 80% methanol (10 mL). The supernatants were pooled, and the volume was made up to 20 mL by methanol. The extracts were stored at −20 °C for 1 week before HPLC analysis.

Phenolic compounds in the methanolic raspberry extracts were analyzed using an Agilent 1100 Series HPLC system (Agilent Technologies) equipped with an autosampler cooled to 6 °C, a DAD (190−600 nm), and an MSD XCT ion trap mass spectrometer fitted with an electrospray ionization (ESI) interface. The compounds were separated on a Synergi 4 μ 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). The separation was executed with mobile phases consisting of A, formic acid/water (2/98, v/v), and B, acetonitrile, with the following gradient: 0−10 min, 5−10% B; 10−22 min, 10−12.4% B; 22−42 min, 12.4−28% B; 42−50 min, 28−60% B; 50−55 min, 60% B; and 55−58 min, 60−5% B. The column was allowed to equilibrate for 5 min between injections (10 μL). The column temperature was held at 40 °C, and the solvent flow rate was 0.25 mL/min.

After UV−vis detection, the effluent was introduced directly, without splitting, to the ESI interface where ionization in negative mode was performed. For identification, some of the samples were, in addition, analyzed in positive mode. The nebulizer pressure was 40 psi; dry gas flow, 10 L/min; dry temperature, 350 °C; and capillary voltage, 3.5 kV. Ions with m/z 100−2000 were measured, with a scan speed of 27000 amu/s. Fragmentation (MS^{2-3}) was carried out in the automatic mode; that is, the two most abundant ions in MS^{1-2} were fragmented. The fragmentation was performed with helium as the collision gas.

The phenolic compounds in the samples were identified based on their UV−vis spectra (190−600 nm), mass spectra, and retention times relative to external standards and comparison with literature reports (*18*, *22*, *39*). The phenolic compounds were classified based on their characteristically UV−vis spectra and quantified by external standards. The anthocyanins were quantified as cyanidin-sophoroside (at 520 nm), ellagic acid, and ellagic acid glycosides as ellagic acid (at 360 nm), ellagitannins as gallic acid (at 260 nm), and quercetin-glycosides as rutin (at 360 nm).

Statistical Analyses. Experimental data were subjected to analyses of variance (ANOVA) and regression analyses by standard procedures using a MiniTab Statistical Software program package (Release 15, Minitab Inc., State College, PA). All analyses were based on triplicate samples from three randomized cultivation blocks, each containing three plants.

RESULTS

Fruit maturation and harvest were advanced in the order of increasing temperature (**Figure 3**). Only the plants in the ambient conditions realized their yield potential of 2.5 kg $plant^{-1}$, while the yield in the controlled temperatures leveled off at about 1.6 kg $plant^{-1}$. This was due to lack of pollinating insects in the phytotron compartments. Therefore, only the flowers pollinated before transfer into the phytotron, produced marketable berries (see **Figure 2**). The berry weight was initially high at all temperatures but decreased steadily with time at 24 and 18 °C, while at 12 °C, and also at ambient temperature, the berry weight remained high during most of the harvest period (**Figure 4**). The main effects of temperature and time of harvest, as well as their interaction, were all highly significant ($p < 0.001$).

Fruit DM, SS, TA, pH, color, and AOC as FRAP, TMA, and TP were all significantly enhanced by increasing temperature during fruit development (**Table 1**). These quality attributes also showed a highly significant ($p < 0.001$) enhancement with progress of the harvest season, and there was also a highly significant interaction of temperature and harvest week on all of these parameters (**Table 1**). All of these effects were mainly a function of a decrease in berry weight with an increase in the temperature and progress of harvest season. As large berries had a higher moisture content than small berries, increasing berry weight resulted in dilution of all water-soluble constituents. Thus, regression analyses revealed a highly significant ($p < 0.001$) negative correlation between berry weight and berry DM and, thereby, also a highly significant negative correlation between the berry weight and the concentration of all of the other fruit quality indicators except AA and pH (**Table 2**). As a result, most of these

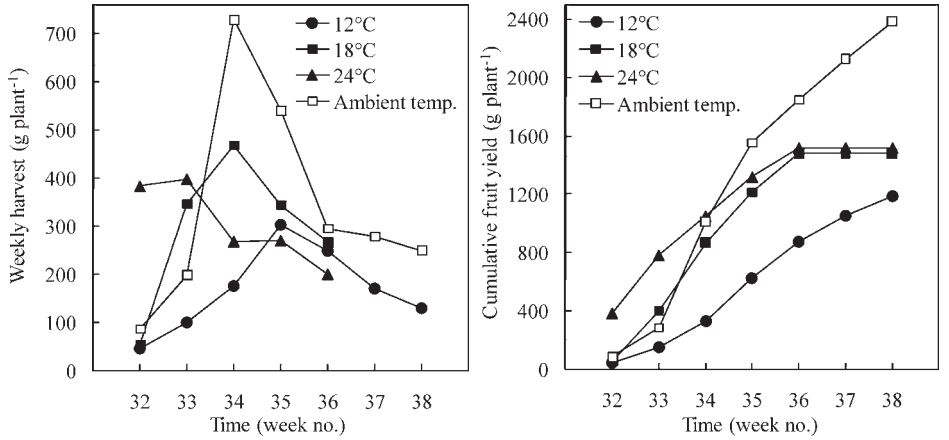

Figure 3. Weekly and cumulative fruit harvests of Glen Ample raspberry plants grown at four temperature regimes as indicated. Values are means of three replicate groups of plants with three plants each.

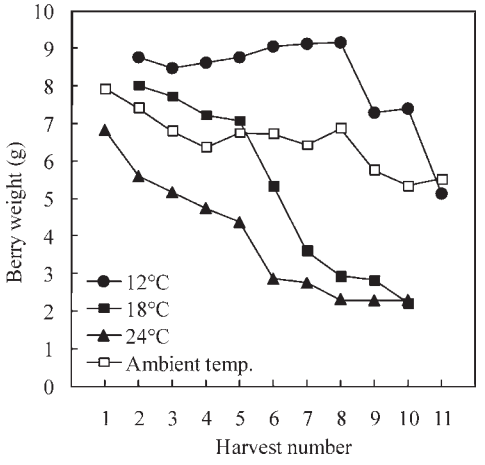

Figure 4. Dynamics of changes in mean berry weight during the harvest period at four temperature regimes as indicated. Values are means of three replicate groups of plants with three plants each.

quality parameters were also highly significantly intercorrelated (**Table 2**). However, when the analysis data were expressed on a dry weight basis, the temperature effects were considerably modified, and in some cases, the trends were even reversed (**Table 3**).

In contrast, pH and AA concentration were not correlated with berry weight, even though they too were influenced by temperature and time of harvest (**Table 1**). The pH increased slightly but significantly ($p = 0.01$) with increasing temperature, while it decreased significantly ($p < 0.001$) with progress of the harvest season, thus yielding a highly significant interaction of the two variables. Similarly, the AA concentration tended to decrease with increasing temperature, but because of a highly significant ($p = 0.004$) interaction of temperature and harvest week, the main effect of temperature did not become significant, whereas the enhancement effect of harvest time progression was highly significant (**Table 1**). Furthermore, when including all harvest times in the ANOVA, also the main effect of temperature was significant ($p = 0.02$). These contrasting effects, opposing the effects of berry size, suggest that the changes in pH and AA concentration were not simply dilution effects related to berry size but were due to other and specific mechanisms.

While there was no significant correlation between AOC (FRAP) and AA concentration, the correlation between the FRAP and the concentration of TP was highly significant ($r = 0.958$, $p < 0.001$), indicating that phenolic compounds were the main contributors to the total AOC of the raspberry fruits. An HPLC analysis of the phenolic profile was therefore performed to estimate the relative contribution of the most important phenolic compounds to the AOC. In accordance with previous studies (*18*,*22*,*39*), anthocyanins and ellagitannins were the major classes of phenolic compounds in raspberry, while ellagic acid glycosides, quercetin glycosides, and flavanols were found in minor amounts. The results in **Table 4** show that while a total of 10 anthocyanins were detected, cyanidin-3-sophoroside and cyanidin-3-(2^{G}-glucosylrutinoside) accounted for about 73% of the total, the former decreasing and the latter increasing significantly with increasing growth temperature. Only cyanidin and pelargonidin type anthocyanins were present. The total anthocyanin concentration increased with increasing growth temperature and progress of the harvest season. In general, there was good agreement between the present results from the HPLC analysis and those with the pH difference method in **Table 1**. In agreement with earlier results with raspberry (*19*, *22*, *23*), by far the most prevalent ellagitannins were lambertianin C and sanguiin H-6, both of which increased significantly (on a fresh weight basis) with increasing growth temperature (**Table 5**). On the other hand, the concentration of free ellagic acid and ellagic acid glycosides was rather low as shown in **Table 6**. Also, the concentrations of total quercetin glycosides in the samples were low, that is, from 0.23 to 0.54 mg rutin equivalents per 100 g of fw (results not shown). Epicatechin and proanthocyanidin dimers and trimers were detected in the samples by MS analysis; however, because of low molar absorptivity and coelution with other compounds, it was difficult to quantify these compounds.

DISCUSSION

The results show that raspberry fruit weight was significantly reduced with an increase in the growth temperature and with progress of the harvest season (**Figure 4**). This has important implications for fruit chemical composition and the concentration of bioactive compounds (**Table 1**). Thus, the correlation analyses presented in **Table 2** revealed a highly significant ($p < 0.001$) negative correlation ($r = -0.862$) between berry weight and DM concentration (i.e., large berries have a higher moisture content). Because the concentration of chemical constituents was expressed on a fresh weight basis in the conventional way, there were also highly significant negative correlations between berry weight and other concentration-dependent quality attributes such as SS, TA, color, and total AOC (FRAP) and its main contributors TP and TMA (**Table 2**). This concurs with the results of Connor et al. (*28*) and Stephens et al. (*40*) and implies that the

Table 1. Effects of Postflowering Growth Temperature and Week of Harvest on Average Berry Weight and Percentages of DM, SS and TA, pH, Color, FRAP, and the Concentrations of L-AA, TMA, and TP of Glen Ample Raspberry[a]

temperature (°C)	week of harvest	berry weight (g FW)	DM (%)	SS (%)	TA (%)	pH	color (515 nm)	L-AA (mg/100 g FW)	FRAP (mmol/100 g FW)	TMA (mg CGE/100 g FW)	TP (mg GAE/100 g FW)
12	33	8.9	10.6	9.5	2.00	2.90	0.40	24.5	2.91	28.9	200.2
	35	8.9	10.9	9.9	1.92	2.96	0.55	24.7	3.48	32.2	235.2
	37	7.6	10.9	9.2	1.89	2.90	0.59	24.9	3.94	32.9	262.1
mean		8.5 a	10.8 bc	9.5 b	1.92 c	2.92 b	0.55 b	24.7 a	3.51 b	31.7 b	236.5 b
18	33	8.0	10.8	9.0	2.30	2.94	0.50	22.2	3.14	30.5	213.0
	35	6.5	11.6	9.8	2.23	2.92	0.62	21.9	4.10	35.5	267.1
	37	2.6	12.9	11.4	3.74	2.86	0.75	28.6	4.21	39.8	275.2
mean		5.4 c	11.9 ab	10.1 ab	2.42 b	2.90 b	0.62 a	24.8 a	3.90 a	35.9 ab	256.6 ab
24	33	6.4	11.2	9.2	2.71	3.06	0.40	21.9	3.42	29.7	215.1
	35	3.7	12.4	10.8	2.32	3.01	0.59	20.9	4.75	39.1	287.6
	37	2.3	12.7	11.5	2.63	2.99	0.70	23.1	4.20	47.3	259.7
mean		4.1 d	12.1 a	10.5 a	2.55 a	3.02 a	0.56 b	22.0 a	4.13 a	38.7 a	254.1 ab
ambient	33	7.7	9.4	8.5	2.32	3.14	0.46	19.1	4.10	34.6	260.0
	35	6.7	11.0	10.1	1.75	2.96	0.54	21.8	3.93	35.2	257.5
	37	5.1	10.9	9.9	1.85	2.94	0.56	24.6	3.90	35.6	261.2
mean		6.5 b	10.4 c	9.5 b	1.97 c	3.01 a	0.52 b	21.8 a	3.98 a	35.1 ab	259.6 a

Probability Level of Significance (ANOVA)

source of variation										
temperature (A)	<0.001	0.01	0.03	<0.001	0.01	0.004	NS	0.002	0.01	0.02
harvest week (B)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A × B	<0.001	0.01	<0.001	<0.001	0.004	0.001	0.004	<0.001	0.05	<0.001

[a] All values are given on a fresh weight (FW) basis. Mean values within the same column followed by different lowercase letters indicate a significant difference ($p < 0.05$) between temperatures. All data are means of three replicates, each with three plants in each temperature treatment.

Table 2. Pearson Correlation Coefficients Between the Various Fruit Quality Attributes Being Investigated[a]

	berry weight	L-AA	pH	color	TP	FRAP	TMA	TA	SS
DM	−0.862***	0.157	−0.048	0.702***	0.690***	0.681***	0.802***	0.664***	0.920***
SS	−0.809***	0.145	−0.166	0.762***	0.593***	0.566***	0.817***	0.573***	
TA	−0.764***	0.059	0.003	0.339*	0.213	0.313*	0.465**		
TMA	−0.745***	0.118	0.002	0.747***	0.632***	0.652***			
FRAP	−0.716***	−0.010	0.150	0.495***	0.958***				
TP	−0.665***	0.085	−0.078	0.640***					
color	−0.632***	0.321*	−0.599***						
pH	−0.028	−0.370*							
L-AA	0.084								

[a] All values based on fresh weight data. ***$p \leq 0.001$, **$p \leq 0.01$, and *$p \leq 0.05$.

effects of temperature and harvest time presented in **Table 1** are mainly due to a higher water content of larger berries. In such cases, presentation on a dry weight basis would be more meaningful.

Berry weight is an important yield component (*32*, *41*) and a useful selection criterion in breeding for high yield potential in red raspberry (*40*, *41*). However, because of the higher moisture content of large berries, fruit phytochemical concentration can easily be compromised, if not carefully monitored, when selecting for larger berries and higher yields (*40*). Similarly, environmental effects on phytochemical concentration involving simultaneous changes in berry size must be interpreted with care. Generally, AOC and the concentration of essential phytochemicals tend to decrease with increasing berry size in both raspberry (*28*, *40*) and other berries such as highbush blueberry (*42*, *43*) and strawberry (*33*, *44*). In blueberry, this is related to the decreasing surface: volume ratio of fruits of increasing size and the prevalence in the skin of anthocyanin pigments and other important phytochemicals (*43*), while in raspberry and strawberry, the association between fruit size and moisture content is the predominant factor (*33*, *44*). Unfortunately, these important implications of fruit size are often overlooked in studies of this kind, and sometimes, fruit size is not even mentioned when such environmental effects are reported (*29*, *30*). The understanding of functional links can then easily be confounded. Therefore, it is essential in such cases that monitoring of phytochemical concentrations is done on a dry weight basis.

Most plant antioxidants are secondary metabolites with important plant protective functions against pathogens and various kinds of environmental stresses. Atkinson et al. (*33*) attempted to increase the concentration of phenolic compounds in two strawberry cultivars by exposing the plants to drought stress. On a fresh weight basis, both free and bound ellagic acid increased significantly when the plants were exposed to drought stress by so-called regulated deficit irrigation, the effect being particularly large when drought was applied during late stages of fruit development. However, drought stress also significantly reduced berry weight so that, after correcting for water content, the ellagic acid

Table 3. Effects of Postflowering Growth Temperature and Week of Harvest on Average Berry Dry Weight and the Concentrations of L-AA, FRAP, TMA, and TP of Glen Ample Raspberries[a]

temperature (°C)	week of harvest	berry weight (g DW)	L–AA (mg/10 g DW)	FRAP (mmol/ 10 g DW)	TMA (mg CGE/ 10 g DW)	TP (mg GAE/ 10 g DW)
12	33	0.94	23.2	2.75	27.3	189.3
	35	0.96	22.7	3.21	29.7	216.8
	37	0.83	23.0	3.63	30.3	241.6
mean		0.91 a	23.0 a	3.25 b	29.3 a	219.2 b
18	33	0.40	20.2	2.90	28.2	196.7.0
	35	0.75	18.9	3.53	30.5	230.0
	37	0.33	22.1	3.25	30.8	212.7
mean		0.51 b	20.5 bc	3.27 b	30.0 a	215.2 b
24	33	0.71	19.7	3.08	26.7	193.1
	35	0.45	16.9	3.84	31.6	232.3
	37	0.29	18.3	3.32	37.4	205.1
mean		0.49 c	18.3 c	3.41 b	31.9 a	210.2 b
ambient	33	0.73	20.3	4.36	36.6	275.8
	35	0.73	19.9	3.60	32.3	235.6
	37	0.56	22.5	3.59	32.9	240.6
mean		0.68 b	20.9 ab	3.85 a	33.9 a	250.7 a

Probability Levels of Significance (ANOVA)

source of variation					
temperature (A)	<0.001	0.003	0.002	NS	<0.001
harvest week (B)	<0.001	0.03	0.005	NS	0.002
A × B	<0.001	NS	<0.001	NS	<0.001

[a] All values are given on dry weight (DW) basis. Mean values within the same column followed by different lowercase letters indicate a significant difference ($p < 0.05$) between temperatures. All data are means of three replicates, each with three plants in each temperature treatment.

concentration was the same in berries of stressed and control plants (*33*). In other words, the effect was an indirect effect of berry size as shown for raspberry in the present study. In this

Table 5. Concentrations (mg/100 g of Fresh Weight) of the Two Most Abundant Ellagitannins in Methanolic Extracts of Glen Ample Raspberry as Affected by Postflowering Growth Temperature and Week of Harvest[a]

temperature (°C)	week of harvest	lambertianin C	sanguiin H-6
12	34	3.7	12.1
	35	4.7	14.2
	36	4.8	15.3
mean		4.6 b	14.4 b
18	34	7.7	16.2
	35	6.8	14.9
	36	7.1	16.7
mean		7.2 a	15.9 ab
24	34	10.2	18.4
	35	7.5	15.4
	36	7.2	15.4
mean		8.3 a	16.3 a

Probability Level of Significance (ANOVA)

source of variation		
temperature (A)	0.002	0.02
harvest week (B)	0.04	NS
A × B	0.004	0.004

[a] The ellagitannins were analyzed by HPLC-DAD-MS and quantified by external standard gallic acid (at 260 nm). Mean values within the same column followed by different lowercase letters indicate a significant difference ($p < 0.05$) between temperatures. All data are means of three replicates, each with three plants in each temperature treatment.

Table 4. Concentrations (mg/100 g of Fresh Weight) of Anthocyanins in Methanolic Extracts of Glen Ample Raspberry as Affected by Postflowering Growth Temperature and Week of Harvest[a]

temperature (°C)	week of harvest	cyd-3,5-diglu	cyd-3-soph	cyd-3-(2^G-glurut)	cyd-3-glu	pg-3-soph + cyd-3-xylrut	cyd-3-rut	pg-3-(2^G-glurut)	pg-3-glu	unknown 1	unknown 2	summed anthocyanins
12	34	0.2	17.2	5.1	3.4	0.9	1.6	0.3	0.2	0.3	0.1	29.4
	35	0.2	17.2	5.3	3.8	1.3	1.8	0.5	0.4	0.6	0.2	31.3
	36	0.2	19.7	6.3	4.1	1.5	1.9	0.6	0.4	0.5	0.2	35.3
mean		0.2 c	18.2 a	5.7 c	3.9 b	1.4 a	1.8 c	0.5 b	0.4 b	0.6 a	0.2 b	32.7 b
18	34	0.8	14.5	9.2	4.8	1.1	4.0	0.6	0.5	0.5	0.3	36.4
	35	1.2	13.9	9.5	5.0	1.3	4.5	0.7	0.6	0.7	0.4	37.8
	36	1.5	14.3	11.5	5.1	1.3	5.0	0.9	0.6	0.7	0.5	41.5
mean		1.1 b	14.2 b	10.0 b	5.0 a	1.2 a	4.5 b	0.7 a	0.6 ab	0.6 a	0.4 b	38.4 a
24	34	1.5	9.1	11.6	5.1	1.1	7.2	0.5	0.7	0.5	0.6	37.9
	35	1.7	8.5	11.7	4.3	1.0	5.9	0.5	0.6	0.5	0.6	35.3
	36	2.3	8.8	13.1	5.2	1.3	7.1	0.6	1.1	0.8	1.0	41.3
mean		1.8 a	8.7 c	12.0 a	4.7 a	1.1 a	6.6 a	0.5 b	0.7 a	0.6 a	0.7 a	37.5 a

Probability Level of Significance (ANOVA)

source of variation											
temperature (A)	<0.001	<0.001	<0.001	<0.001	NS	0.004	NS	0.005			
harvest week (B)	0.02	0.01	<0.001	0.03	<0.001	NS	<0.001	0.006	0.003	0.001	0.001
A × B	NS	0.01	NS	0.03	0.002	NS	0.04	NS	0.04	0.006	NS

[a] The anthocyanins were analyzed by HPLC-DAD-MS and quantified by external standard cyanidin-3-sophoroside (at 520 nm). Mean values within the same column followed by different lowercase letters indicate a significant difference ($p < 0.05$) between temperatures. All data are means of three replicates, each with three plants in each temperature treatment. Abbreviations: cyd, cyanidin; pg, pelargonidin; diglu, diglucoside; soph, sophoroside; glu, glucoside; rut, rutinoside; and xyl, xylosyl.

Table 6. Concentrations (mg/100 g of Fresh Weight) of Ellagic Acid Glycosides and Free Ellagic Acid in Methanolic Extracts of Glen Ample Raspberry as Affected by Postflowering Growth Temperature and Week of Harvest[a]

temperature (°C)	week of harvest	ellagic acid pentoside 1	ellagic acid pentoside 2	ellagic acid	methyl ellagic acide pentoside	ellagic acid 4-acetylxyloside	total ellagic acid glycosides (including ellagic acid)
12	34	0.18	0.19	0.12	0.07	0.08	0.64
	35	0.18	0.20	0.14	0.06	0.07	0.64
	36	0.16	0.19	0.15	0.05	0.06	0.61
mean		0.17 c	0.20 c	0.14 c	0.06 b	0.06 b	0.63 c
18	34	0.26	0.29	0.21	0.11	0.12	0.99
	35	0.26	0.30	0.26	0.10	0.11	1.03
	36	0.28	0.35	0.32	0.11	0.11	1.17
mean		0.27 b	0.31 b	0.26 b	0.10 b	0.11 b	1.06 b
24	34	0.55	0.59	0.39	0.22	0.27	2.03
	35	0.45	0.51	0.40	0.17	0.20	1.73
	36	0.46	0.58	0.48	0.17	0.22	1.91
mean		0.48 a	0.55 a	0.42 a	0.19 a	0.22 a	1.86 a

Probability Level of Significance (ANOVA)

source of variation						
temperature (A)	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
harvest week (B)	NS	0.005	NS	NS	NS	0.04
A × B	NS	NS	NS	NS	NS	NS

[a] The ellagic acid derivatives were analyzed by HPLC-DAD-MS and quantified by external standard ellagic acid (at 360 nm). Mean values within the same column followed by different lowercase letters indicate a significant difference ($p < 0.05$) between temperatures. All data are means of three replicates, each with three plants in each t emperature treatment.

connection, it is important to notice that in the present experiments with raspberry, the water vapor saturation deficit was kept constant across the range of temperatures. This gives a constant rate of transpiration independent of temperature and, hence, rendering the temperature effects specific and not confounded by associated drought effects.

While drought stress failed to induce specific changes in fruit chemical composition (*33*), the prospects for activation of specific biosynthesis pathways may be better for manipulation of the light environment. Thus, it is well-known that anthocyanin biosynthesis is induced by UV light in both fruits and leaves as an important photoprotective mechanism, especially at low temperature (*45*). Also, low temperature is shown to induce the accumulation of the enzyme phenylalanine ammonia lyase (PAL) in *Arabidopsis* in a light-dependent manner (*46*). PAL acts at the branch point between primary and secondary metabolism and so has an important regulatory function in the biosynthesis of many of these phenolic compounds. Because the activity of PAL is also under the control of the phytochrome pigment involved in photoperiodism (*47*), it is also likely that photoperiod might influence the berry content of phenolic compounds.

Generally, on a fresh weight basis, chemical fruit composition in the ambient temperature treatment with fluctuating day/night temperature did not vary significantly from those of the corresponding constant 12 and 18 °C treatments (**Table 1**). On a dry weight basis, however, FRAP and TP were significantly higher in berries from the ambient temperature than in the lower constant temperature treatments (**Table 3**). In addition, the highest TMA value was also found under the ambient condition ($p = 0.067$). Although these data do not allow any definite conclusions, they suggest that a fluctuation in the temperature may enhance the concentration of secondary metabolites in raspberry fruits, as compared with constant temperatures.

In contrast to most other analysis parameters, the AA concentration was not negatively correlated with berry fresh weight (**Table 2**) but tended to increase with decreasing growth temperature, even on a fresh weight basis (**Table 1**). On a dry weight basis (**Table 3**), there was a stepwise and highly significant ($p = 0.003$) increase in AA concentration as the temperature was lowered from 24 to 12 °C (including the ambient condition). In the Nordic countries, it is a long-standing anecdotal notion that fruit and vegetable commodities grown under the special temperature and light conditions at high latitudes may be particularly rich in AA. There is, however, little experimental evidence to support this, although Wang and Camp (*30*) found that the AA concentration in strawberry increased with decreasing day and night temperature. However, in the present experiments with raspberry, there was a specific increase in the AA concentration at low temperature, which more than compensated for the corresponding large increase in berry size and water content. Similarly, Remberg et al. (*43*) found a highly significant positive correlation between berry weight and AA concentration of seven red raspberry cultivars.

The concentrations of potentially beneficial nutritional phytochemicals listed in **Table 1** were in good agreement with previous analyses reported for raspberry (*1*, *27*, *28*, *40*). The results confirm that raspberries are a rich source of antioxidants and that phenolic compounds are the main contributors (*23*−*25*), while AA constitutes a minor part of the total (*7*). The profile of phenolic compounds listed in **Tables 4**−**6** shows that anthocyanins and ellagitannins were the main contributors to the total AOC (*1*). The anthocyanin concentration is known to vary much among raspberry cultivars (*1*). Glen Ample is reported to have midrange values (*19*, *23*) as confirmed by the present results (35 mg/100 g FW), while some late-maturing Spanish cultivars have been reported to have particularly high values with up to 116 mg/100 g FW (*21*). Keeping in mind the large effect of temperature on berry size (**Figure 4**), it seems likely that this might be an indirect effect of the hot Spanish summer on late-maturing cultivars.

The marked positive effect of low growth temperature on berry size (**Figure 4**) is apparently an important contributor to the large

yields of Glen Ample raspberry in the cool Nordic climate (*32*). However, the associated increase in moisture content has a dilution effect on berry AOC and the concentration of anthocyanins and TP compounds when expressed on a fresh weight basis in the conventional way (**Table 1**). However, in spite of this dilution effect, the concentration of vitamin C actually increased at low temperature, even on a fresh weight basis, indicating that low temperature has a specific positive effect on the concentration of this important vitamin. The results demonstrate that large fruits and high fruit AOC have different growth temperature optima; therefore, the selection for both attributes will require compromise. However, because large fruits are an important quality attribute in itself and because the negative effect of low temperature on chemical berry constituents was rather inconsistent and nonexisting for some constituents, a relatively low temperature of 12−18 °C would be an optimum temperature compromise for protected raspberry cultivation. Clearly, the growth temperature has significant and contrasting effects on the concentration of a range of bioactive compounds in raspberry fruits. Especially interesting is the positive effect of decreasing temperature on AA concentration, which more than compensated for the associated dilution effect of large berries.

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