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Fruit quality in strawberry (*Fragaria x ananassa* Duch. cv. Korona) at three times during the season and with two fertilizer strategies

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Summary

Effects on fruit quality of preplant fertilization and fertigation in strawberry were studied in a field experiment established August 2003 in South East Norway. Half the field was fertilized at planting and fertigated with a low nutrient rate from spring 2004 (T1), while the second half was unfertilized at planting but fertigated with a relatively high nutrient rate from spring 2004 (T2). Berries were harvested three times per week during the first harvesting year. Samples of berries from early season, mid season and late season were analysed for soluble solids, pH, titratable acidity, colour, vitamin C, antioxidant activity (FRAP) and mineral concentration. The results were correlated to leaf mineral concentration in dry matter. T1 gave larger and darker berries compared to T2. The other quality parameters and mineral content in strawberry fruits were not significantly influenced by the two fertilizer strategies. Soluble solids, pH, titratable acidity and colour varied significantly through the season, while vitamin C and antioxidant activity (FRAP) was unaffected by fertilizer treatment, berry size and time of season. The results gave a general view of the quality parameters in the strawberry cultivar 'Korona'.

Introduction

A number of preharvest factors have been found to influence on postharvest quality of berry crops, including genetics, climate, water management and other cultural practices (PRANGE and DEELL, 1997). Genotype present the main variation in fruit composition, and the content of flavonoids and phenolic compounds are found to vary significantly between different berry species, with certain similarities within family and genera (HÄKKINEN et al., 1999). Strawberries contain high levels of antioxidant compounds (HALVORSEN et al., 2002), with a considerable variation between different cultivars (DAVIK et al., 2006). Both KALLIO et al. (2000) and DAVIK et al. (2006) found differences between cultivar in the sugar and acid composition in strawberry. Variation in climate at different geographical sites and between years was also found to influence on the quality factors in these experiments. Cultural practices such as ground cover and mulching affect fruit quality as well (NEUWEILER et al., 2003; MOOR et al., 2005; ANTTONEN et al., 2006), and mulch type was suggested to affect fruit quality through changes in the soil moisture (NEUWEILER et al., 2003). The results from HANSEN (1995) and KIRNAK et al. (2003) also indicated that water stress reduced fruit size and increased soluble dry matter.

Application of fertilizer has a great impact on various yield and fruit quality parameters in strawberry, and the major point of interest has been berry size, saleable yields and the content of soluble solids. As it has become well known that a diet rich in fruits and vegetables reduce the risk of developing cardiovascular diseases, cancer and a number of chronic diseases, fertilizer effects on the antioxidant content in assorted horticultural crops has also been reported (JEPPSON, 2000; LEE and KADER, 2000; ANTTONEN et al., 2006). Strawberries contain high levels of antioxidant compounds (HALVORSEN et al., 2002), and although genotype has the greatest impact on antioxidant content in berries, cultivation methods that may improve the quality and health benefits of strawberries for fresh consumption is of current interest. The aim of the present study was to establish possible effects from time of fertilizer application on selected fruit quality parameters in the strawberry cultivar 'Korona'. In addition to the fertilizer effects, the experiment gave a good opportunity to show quality parameters in fruits of the strawberry cultivar 'Korona' at three harvesting times. Absolute and relative fruit growth rate varies according to rank order in the cyme (FORNEY and BREEN, 1985), and effects on fruit quality of harvest dates are documented (SJULIN and ROBBINS, 1987; ANTTONEN et al., 2006). Ripe berries from early season, mid season and late season were therefore analysed in the present trial.

Material and methods

The trial was conducted from 2003 to 2004 at Apelsvoll Research Center division Kise, Norway (60º40'N, 10º11'E), on a morainic loam soil. The field was planted on August 22nd 2003 using certified rooted plug plants. Plants of the cultivar 'Korona' were planted in double rows, on low ridges covered with black polyethylene mulch. Plant spacing was 25 cm within the row, 40 cm between the double rows and 160 cm between ridges. The whole field was equipped with a pressure compensated drip irrigation system connected to a fertilizer injector. The tubes were placed under the polyethylene mulch in the centre of the ridges. Before planting, a compound fertilizer equivalent to 500 kg ha^{-1} of 6-5-20 NPK with micronutrients (Fullgjødsel® NPK 6-5-20 Mikro) was applied to half of the rows (treatment 1; T1) (Tab. 1). The rows in the latter half were not fertilized prior to planting (treatment 2; T2). Fertigation was carried out in both treatments twice a week from May to August in 2004 and 2005, using Calcinit™(15.5% N, 19.0% Ca) and Super-ba™Rød (7-4-22 NPK + micronutrients). All fertilizers were from Yara. In the first cropping year, T1 and T2 were fertigated with different amounts of nutrients in the same volume of water. In T2 the EC was kept constant at 2.4 mS cm^{-1} and the ratio between Calcinit™and Superba™was 5:7, to get a total nutrient composition similar to T1. T1 was fertigated concurrently, but EC was kept on 1.6 mS cm^{-1}. At the end of the first cropping season, the total amount of water and nutrients that had been applied was similar for both treatments for the first two years. The amount of N added during 2003 and 2004 was 80 kg ha^{-1} in total, which is the recommended fertilizer amount per year for 'Korona' in Norway. The experimental design was a

Tab. 1: Summary of the fertilizer treatments in the trial presented as kg N ha^{-1} $year^{-1}$.

Treatment	2003	2004	(2005)
T1	30[1)]	50[2)]	80[2)]
T2	0	80[2)]	80[2)]

[1)] Broadcasted compound fertilizer
[2)] Fertigation

randomized complete block with three replicates, each replicate counting 200 plants.
First fully developed leaves for dry matter analyses were sampled at the same times as the berry samples were harvested, dried at 70ºC and ground. Berries were harvested three times per week on 50 plants per plot during the harvesting period in 2004 from June 21st to July 19th. Samples of berries from early season, mid season and late season were registered and frozen at -18ºC for quality analyses, while sub-samples were dried for dry matter analyses.

Dry matter analyses

Leaf macronutrient concentration in dry matter were analysed after sulphuric/hypochloric acid digestion (ALLEN et al., 1974), using a Skalar autoanalyser for N and P, flame photometry for K and atomic absorption spectrophotometry for Ca and Mg. The same method was used when analysing mineral concentration of the berries.

Fruit quality analyses

Colour

For colour analyses the juice was diluted to a 5 % solution by adding distilled water. The absorbance was measured at 515 nm using a spectrophotometer (Shimadzu UV mini 1240, Shimadzu Corporation, Kyoto, Japan).

FRAP assay

Fruits were homogenised in a food processor, and 3 g of the homogenised material was dissolved in 30 ml of methanol. Three replicates were prepared from each sample. The bottles were flushed with nitrogen before closing, the samples were then mixed and sonicated on a water-bath at 0 °C for 15 min. The extracts were stored at -20 °C until analysis.
Three samples were centrifuged at 3250 g for 4 min at 4 °C. The concentration of total antioxidants was measured in triplicates of the supernatant.
The Ferric Reducing Ability of Plasma (FRAP) assay of BENZIE and STRAIN (1996) was used with minor modifications (HALVORSEN et al., 2002). A Konelab 30i (Termo, Finland) was used to measure the antioxidant activity. The measurements were performed at 600 nm. An aqueous solution of 500 µM $FeSO_4$ x 7 H_2O was used for calibration of the instrument.

Vitamin C

50 g of frozen material was made up to 150 g using 1 % oxalic acid. The material was homogenized for 1 minute and filtered through a Schleicher and Schuell filter (520 B1/2, folded). The samples were then filtered through an activated Sep-pack C18 filter (Waters) and a Millipore filter (0,45 µm) before injection. Prior to use the Sep-pack filter was activated using 5 ml of methanol and 5 ml of ultra pure water. The first 2 ml of the analytic sample was discarded. The HPLC analysis was performed according to WILLIAMS et al. (1973) using an Agilent system comprising HP1100 liquid chromatograph, auto sampler and UV detector (Agilent Technologies, Oslo, Norway). Monitoring of the chromatography and data processing were performed by means of Chemstation software. The separation was conducted on a 250 x 4.6mm Zorbax SB-C18 5 µm column (Agilent Technologies, Oslo, Norway). The mobile-phase was 0.05M KH_2PO_4 for isocratic elution at 25 ºC. The flow was 1 ml min^{-1}. The injection volume was 5 µl and the time was set to 5 min. L-ascorbic acid was measured at 254 nm.

Sugars, acids and total dry matter

Samples of juice were prepared for analyses of soluble solids and tritratable acidity. Soluble solid concentration was determined by a digital refractometer (Atago refractometer model PR-100, Atago Co, LTD. Tokyo, Japan), the titratable acidity by a radiometer end-point titrator (Radiometer End Point Titration System, ETS 822, Copenhagen, Denmark) and calculated as citric acid. Dry matter was determined by drying approximately 5 g of homogenized material for 24 h at 104 ºC and weighing after stabilizing at room temperature in a dry container.

Statistics

All data were subjected to analysis of variance (ANOVA) using MiniTab® Statistical Software (Release 14). Significant differences between treatments were identified at the 5% probability level. For correlations, the Pearson correlation coefficient (r) was calculated.

Results

Preplant fertilization in August 2003 (T1) was positive for yield and berry size (OPSTAD et al., unpublished), but the fertilizer treatments in this trial had only minor effects on the quality parameters recorded. Only fruit colour was significantly darker in T1 (P=0.002),

Tab. 2: Quality parameters in strawberry fruits with two fertilizer strategies at three harvesting times during 2004.

		Ref		pH		% Acid		Colour (5%)		Vitamin C (mg 100 g^{-1} fresh weight)		FRAP (µmol g fresh $weight^{-1}$)		Berry size (gram $berry^{-1}$)	
Time	Date	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Early season	25.06.04	11.37	11.80	3.60	3.61	0.84	0.84	0.07	0.06	57.16	54.36	17.53	18.09	36.43	31.90
Mid season	05.07.04	10.20	10.63	3.41	3.44	0.90	0.90	0.12	0.09	55.39	57.26	18.05	17.84	18.13	13.30
Late season	15.07.04	12.00	12.47	3.48	3.48	0.93	0.98	0.09	0.07	55.99	57.39	18.57	19.12	9.00	9.00
Analysis of variance (P-value)															
Source	Treatment	0.058		0.604		0.527		0.002		0.872		0.723		0.001	
	Time	<0.001		<0.001		0.006		<0.001		0.728		0.566		<0.001	
	Treatment x Time	0.997		0.727		0.604		0.264		0.137		0.909		0.020	

while T2 tended to have a higher concentration of soluble solids (P=0.058) (Tab. 2). Soluble solids, pH, acidity and colour all varied with time of season. pH was highest in early season and lowest in mid season, while acidity was increasing through the season. Antioxidant content was similar in both treatment and at all sampling times, while berry size was affected by treatments until the last sampling date as well as with sampling time (Tab. 2). This indicates that vitamin C and FRAP was unaffected of both treatment, sampling time and berry size. Neither the concentration of N, P, K, Ca or Mg in dry matter of strawberry fruits showed any variation with time or treatment (Tab. 3). N, K and Ca concentration in leaf dry matter varied significantly between the three sampling times, while concentration of Ca and Mg was affected by treatments (Tab. 4). There were no correlations between fruit quality parameters, mineral concentration in berries or mineral concentration in leaves or sap (data not presented).

Discussion

The lack of fertilizer response on fruit quality in strawberry in the present trial is probably due to the small difference in fertility level in T1 and T2 in the cropping year. Fruit quality may not be easily affected by moderate fertilizer rates, and ALLEYNE and CLARK (1997) and MINER et al. (1997) found no fertilizer effects on various fruit quality components.

The fertilizer application timing in the present trial significantly affected yield parameters, probably by augmenting flower differentiation in autumn (OPSTAD and SØNSTEBY, unpublished). The only statistical significant fertilizer effects on fruit quality was, however, concerning fruit colour, and the preplant fertilized plots receiving the lowest fertilizer rate during the harvesting season had the darkest fruit colour. Application of 50 g N per grape vine increased the level of anthocyanins and the density of colour of grape must, compared to no fertilizer N application (DELGADO et al., 2004). Application of 200 g per vine resulted in a colour density in-between the control and 50 g treatment. The fertilizer rate applied in our trial in T1 in 2004 may therefore have been optimal for colour development. Alternatively, the positive effect on fruit colour in T1 may have been due to translocation of N from the autumn fertilization (ARCHBOLD and MACKOWN, 1995) as the fertilizer applications in T2 was quite moderate. Application of K had no effect on colour density in grape (DELGADO et al., 2004).

In our trial, plants fertigated with the highest amount of fertilizer during the harvesting season (T2) tended to yield fruits with a higher content of soluble solids, corresponding to the results of WANG and LIN (2002). Also NESTBY (1998) registered higher sugar content when applying 124 kg N ha^{-1} than at lower N levels. The opposite was found by VOTH et al. (1967) and HANSEN (1995), were the lowest fertilizer rate resulted in the highest soluble solids content. Other trials have, however, found no fertilizer effects on content of soluble solids (ALLEYNE and CLARK, 1997; MINER et al., 1997; JEPPSON, 2000).

All of the measured quality parameters except content of antioxidants and vitamin C varied between sampling times. The content of soluble solids decreased from early season to mid season, and maximized at the end of season. Also MINER et al. (1997) and HANSEN (1995) found a higher concentration of total and soluble solids in berries at the late picking dates, and HANSEN (1995) suggested this to be an effect of increased access to assimilates due to removal of the

Tab. 3: Mineral concentration in strawberry fruits with two fertilizer strategies at three harvesting times during 2004.

		N		P		K		Ca		Mg	
Time	Date	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Early season	25.06.04	0.933	1.103	0.197	0.237	1.260	1.400	0.127	0.123	0.097	0.107
Mid season	05.07.04	1.017	1.070	0.207	0.217	1.260	1.363	0.107	0.117	0.097	0.103
Late season	15.07.04	1.017	0.983	0.213	0.213	1.337	1.280	0.113	0.103	0.097	0.097
Analysis of variance (P-value)											
Source	Treatment	0.312		0.185		0.293		0.949		0.231	
	Time	0.839		0.939		0.944		0.703		0.647	
	Treatment x Time	0.409		0.384		0.353		0.887		0.647	

Tab. 4: Mineral concentration in strawberry leaves with two fertilizer strategies at three sampling times during 2004.

		N		P		K		Ca		Mg	
Time	Date	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Early season	25.06.04	2.463	2.510	0.290	0.327	1.367	1.410	0.677	0.653	0.237	0.233
Mid season	05.07.04	1.977	2.047	0.277	0.280	1.300	1.227	0.647	0.537	0.230	0.210
Late season	15.07.04	1.673	1.913	0.280	0.313	1.437	1.503	0.610	0.460	0.240	0.213
Analysis of variance (P-value)											
Source	Treatment	0.151		0.456		0.837		0.023		0.010	
	Time	<0.001		0.324		0.045		0.040		0.116	
	Treatment x Time	0.550		0.441		0.589		0.365		0.227	

strongest sinks by picking of the primary berries. This could also explain the decrease in soluble solids in berries harvested mid season in the present trial, when a large number of berries were competing for assimilates. These differences between sampling time of ripe berries suggest that time of season must be considered when comparing fruit quality in relation to varieties or cultivation conditions. A decrease in shelf life from early to late season has also been found (OPSTAD et al., unpublished), as well as differences in fruit maturation related to fertilizer treatments (OPSTAD and SØNSTEBY, unpublished). Ideally, fruit samples should therefore be defined in relation to fruit rank order before comparing fertilizer and harvest time effects on fruit quality.

A significant negative relationship between fruit size and total antioxidant capacity has been observed for strawberry cultivars (DAVIK et al., 2006) and high-bush blueberry (*Vaccinium corymbosum*) cultivars (REMBERG et al., 2006). This was explained by the surface to volume ratio, as the peel fraction of fruits have a significantly higher antioxidant capacity compared to pulp (GUO et al., 2003). In this study, berry size decreased during the season without any significant influence on antioxidant activity. WANG and LIN (2000) found a linear relationship between the content of anthocyanins and antioxidant activity measured by ORAC assay (Oxygen Radical Absorbance Capacity), but in our trial the significantly darker fruit colour in the T1 treatment was not associated with antioxidant activity measured by FRAP assay. Thus, other components than anthocyanins, like ellagic acid (ANTTONEN et al., 2006), contribute significantly to the antioxidant capacity in strawberries. The vitamin C content has also been found to correlate negatively with fruit size where smaller fruits contained more vitamin C compared to larger fruits (MOOR et al., 2004), but in this study the vitamin C content was not influenced by the berry size. MOOR et al. (2005) found that vitamin C content in strawberry is not easily influenced by cultural practises, and in tomato fruits, fertilization using electrical conductivity of 2 and 5 mS cm^{-1} had no significant effect on antioxidant activity (WOLD et al., 2004). In contrast, the same trial demonstrated a significant increase in dry matter, soluble solids and titratable acids with higher electrical conductivity. The lack of correlations with berry size in our trial, could possibly be due to unknown effects of sampling time that is not correlated to berry size.

The concentration of macro nutrients in berries analysed in this trial was not affected by neither fertilizer treatments nor sampling time, while a statistical significant effect was found of sampling time in respect to mineral elements in leaf dry matter. Only concentration of Ca and Mg in leaf dry matter was affected by the fertilizer treatments. Surprisingly, the fertilizer treatment where the highest fertilizer amount was applied during the harvesting season (T2), showed a decrease in concentration of all mineral elements in fruits from early to late season, while in the preplant fertilized treatment (T1) there was an increase in NPK concentration from early to late season. These changes were, however, not significant. ARCHBOLD and MACKOWN (1995) found that fruit under development was a greater sink for stored fertilizer N acquired in the months before cropping than for newly absorbed N, and may explain these tendencies in our results. The concentration of all macronutrients in the berries in our trial was lower than the concentration found in dry matter of three strawberry cultivars and clones in Florida (ALBREGTS and HOWARD, 1980). This is probably due to differences between cultivars or climatic factors, as the fertilizer treatments used in our trial are according to recommended fertilizer rates. N concentration in fruit dry mass in blackberry (*Rubus* L., subgenus *Eubatus*) was unaffected by fertilizer timing, and only the control was significantly lower than other fertilizer treatments (ALLEYNE and CLARK, 1997).

In conclusion, moderate fertilizer rates applied in late autumn or during the harvesting season resulted in only marginally different effects on fruit quality parameters, and only fruit colour was significantly affected by the fertilizer treatments. Although significant differences between harvest date was found for berry size, soluble solids, pH, titratable acids and colour in fruits, and N, K and Ca concentration in leaves, no effects on antioxidant activity or fruit mineral concentration was found. Other factors probably affected fruit composition more than application of fertilizer.

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