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Effect of Fruit Order, Polyethylene Mulch Colour and Early Forcing on the Fruit Quality of Tunnel-grown Strawberry cv. 'Korona'

R. Nestby[1)], M. J. Anttonen[2)], M. Verheul[3)] and R. Karjalainen[2)]
([1)]The Norwegian Crop Research Institute, Kvithamar Research Centre, Stjørdal, Norway, [2)]University of Kuopio, Institute of Applied Biotechnology, Kuopio, Finland and [3)]The Norwegian Crop Research Institute, Særheim Research Centre, Norway)

Summary

The effects of fruit order, polyethylene mulch colour and early forcing on the biochemical compounds (BCs) vitamin C (L-ascorbic acid), percentage soluble solids (°Brix), total acids, total anthocyanins, total phenolics and ellagic acid of strawberry (*Fragaria* x *ananassa* cv. 'Korona') were examined. The study was conducted in three consecutive years on fruit taken from a split-plot design involving a tunnel. There were large differences in fruit season and yield between the first and second fruiting years, reflected in the differences between levels of examined BCs of fruit orders, which ranged differently. The differences observed in °Brix and total acids were not sufficient to have a major effect on fruit taste. Using white polyethylene mulch (WM) increased the °Brix-value in one of the three years of the study and vitamin C in all years compared to brown polyethylene mulch (BM). Total acids were completely unaffected by mulch-colour during the whole course of the study. The contents of total phenolics, total anthocyanins and ellagic acid were examined only for the last year, and no significant effect of mulch colour for total anthocyanins was observed, while ellagic acid and total phenolics increased on WM compared to BM. Forcing made the plants fruit four or seven days earlier in the first and second year, respectively, but had no influence on the examined BCs. However, forcing strongly reduced fruit size and increased the percentage of malformed fruits in one year.

Key words. °Brix – total acids – total phenolics – vitamin C – total anthocyanins – ellagic acid – yield parameters

Introduction

Strawberries are rich in total antioxidants and are thus important for human health (Halvorsen et al. 2002). However, the quality and quantity of these compounds are affected by genetic, environmental and agricultural factors (Kallio et al. 2000; Anttonen and Karjalainen 2005). Fruit order affected fruit fresh weight, gloss, firmness and shelf life. Concerning °Brix there were interactions between fruit position in the canopy and fruit order. Furthermore, taste, fruit firmness and °Brix varied between individual fruits (Osman and Dodd 1994a, b; Døving and Måge 2001).

Interactions between plant development and climate and seasonal variations could affect fruit quality, in the same way as interaction between freezing tolerance and climate in strawberry (Nestby et al. 2001) and as seasonal variations do in the case of tomatoes (Slimestad and Verheul 2005).

Shading experiments with strawberries suggest that compensation for reduced assimilate supply occurs, if the light integral (LI) does not drop below 2.42 MJ PAR m^{-2} d^{-1} (Awang and Atherton 1995; Fletcher et al. 2002). Mulch and rowcover of spunbound polypropylene (fleece) for early forcing, both affect light conditions and temperature. White polyethylene mulch (WM) raised the average air-temperature in the leaf canopy by 3–4°C compared to that with no mulch (Lieten 1993). To achieve the highest possible net photosynthesis a temperature of 15–25°C is optimum, while the maximum photosynthetic-value was achieved at 20°C in the period of first unfolding leaf and under fruit development, and at 15°C during flowering and growth of runners (Elsacker et al. 1989; Yun and Yoo 1992).

Generally, exposure to temperatures higher than 35 °C reduced plant growth (Renquist et al. 1982; Hellman and Travis 1988), and fruit yield (Hellman and Travis 1988). Little information has been available describing the effect of temperature on nutritionally important compounds in strawberries (Rariden et al. 1993). However, recently it was shown that strawberry fruits had a lower antioxidant capacity when grown at 18°C/12°C (day/night temperature) than at 30°C/22°C, respectively (Wang and Zengh 2001).

The aim of these experiments was to examine,

1) if there are differences in some quality parameters between fruits of a single plant linked to fruit order,
2) if mulch colour, besides its effect on temperature and light, could affect some important fruit quality parameters, and
3) if pre-bloom forcing could have an effect on these.

Fruit quality, as defined in this work, include both classical fruit quality parameters as °Brix and total acids, and other compounds important for the quality such as vitamin C, total phenolics, total anthocyanins and ellagic acid.

Materials and Methods

Location and cultural system

The experiments were carried out in the field at Stjørdal, Norway (Latitude 63 ° 29 '; longitude 10 ° 52 '; 38 m a.s.l.) in 2003, 2004 and 2005. The soil was a poorly drained silt loam overlaying silty clay loam with an almost flat slope, containing 8.3 % organic matter in the plough layer. Flower bud initiated 'Korona' strawberry, was planted on 2 July 2003 on 25 cm high beds mulched with polyethylene foil (Norfolier® AS, Norway). The centres of the beds were 140 cm apart, with two plant rows on each bed. Rows were planted 20 cm apart with 25 cm plant spacing within each row, giving 5714 plants per 1000 m^2. Immediately after planting a tunnel, 7.5 m wide, roofed with standard polyethylene foil (Haygrove® England) was placed over the field with five beds within the tunnel. The two first weeks after planting the plants were watered with overhead sprinklers. Later that year and in 2004 and 2005, plants were drip irrigated with a balanced macro- and micro- nutrient solution, regulated by a 'Flori' soil moisture sensor (Netafim® USA). From 10 October 2003 to 20 April 2004 and from 10 October 2004 to 20 April 2005 the field was covered with fleece ('Agryl P30 vinter' Norway) for freezing protection. In 2004 and 2005 the tunnel was roofed on 20 April.

Experimental design and methods of recording

Experiments were carried out in a tunnel with two treatments in a split plot design of six replications placed across the rows of the tunnel. Treatments were

1) Forcing on large plots (120 plants harvested) randomised within replication,
   a: from 2. July 2003 and from 20. April 2004, to 5 % flowering, and
   b: no forcing;

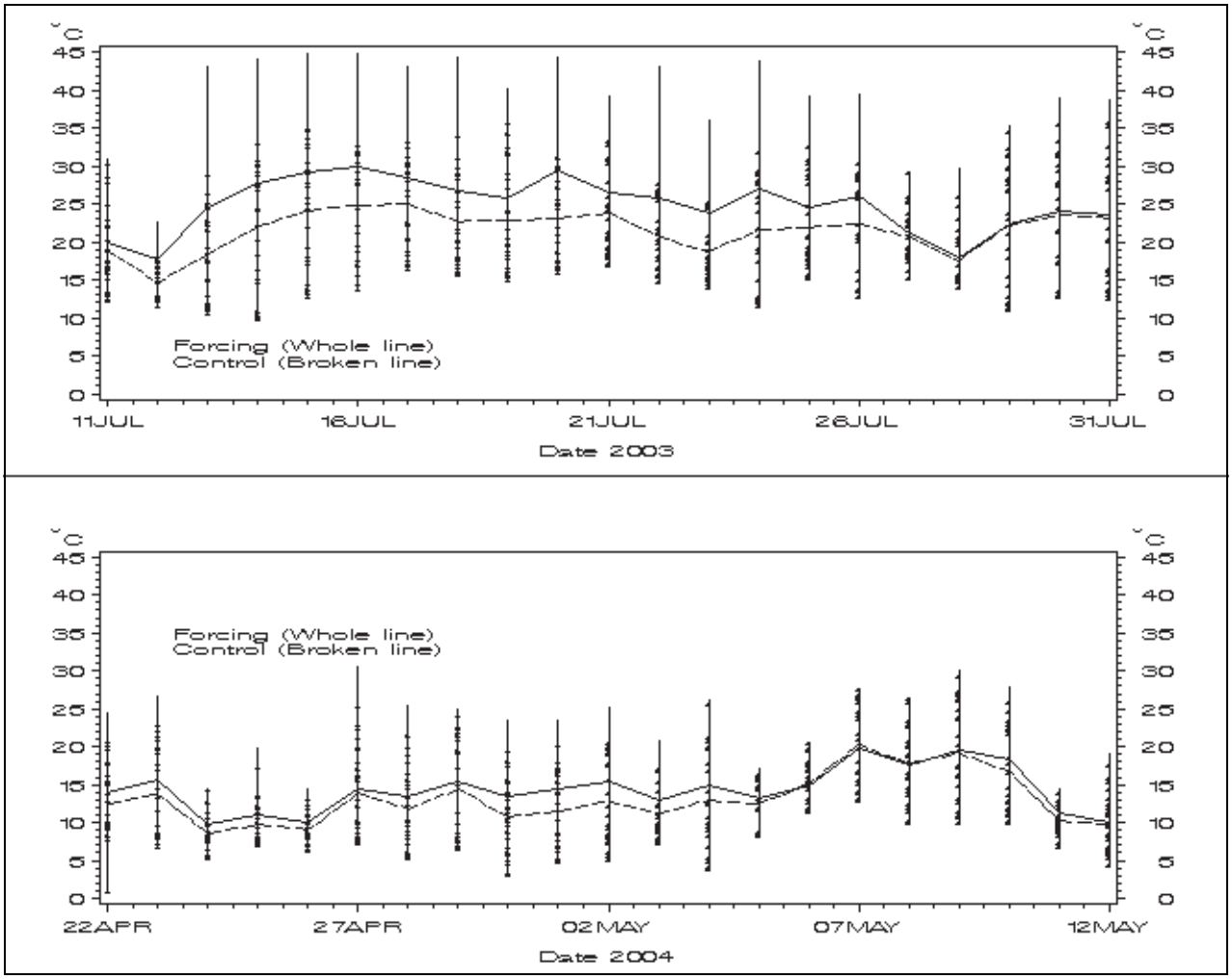


Fig. 1. Effect of early forcing on the daily average, high and low temperatures in the leaf canopy of 'Korona' strawberry. (Observation period: 2003–2004; Site of temperature sensor: 5 cm above ground).

2) Mulch on small plots (60 plants harvested) randomised within large plots,
   a: WM (white polyethylene mulch),
   b: BM (Brown polyethylene mulch).

Temperatures (°C) were monitored 5 cm above the mulch within the plant canopy in both mulch types and under the fleece. The photosynthetic active radiation (PAR; μmol m^{-2} s^{-1}) was measured at ground level inside and outside the tunnel in 2003 and 2004 and transformed to MJ m^{-2} d^{-1}. Forcing increased the air temperature in the leaf canopy strongly in 2003, and the daily maximum could be 10 °C higher than control, reaching 46 °C (Fig. 1). Also in 2004 there was a temperature effect of forcing, but the daily maximum was about 10 °C lower than the previous year. In the presence of mulch the air temperature was generally higher with BM than with WM in 2003 (Fig. 2). In contrast, in 2004, except in spring when the leaf mass was still small, and especially during the warm weather in August, temperatures over WM could be 2–3 °C higher than for BM.

Fruits were harvested in 2003 as an autumn crop and in 2004 and 2005 as mid-summer crops. During fruit development there was less light in 2003 than in 2004 and subsequently in 2005, because of the late harvest season in 2003 (start: mid-August) compared with 2004 (start: 25 June) and 2005 (start: 5 July). As well as the fruit yields (kg 1000 m^{-2}), the percentages of fruit in the classifications large saleable fruits (diameter ≥25 mm) and small saleable fruits (diameter ≤25 mm), rot (grey mould), mildew, malformed and rest (damaged due to birds, snails etc.) were recorded. Fruit size was calculated in grams per fruit on the basis of 25 randomly selected fruits at each harvest date, weighing mean fruit size according to fruit yield at each harvest date. The relative differences in chlorophyll contents of leaves were recorded using a Hydro N-tester (Yara® Norway) at anthesis in the first year, and at first fruit harvest in both successive years. For analyses of fruit quality, caution was taken to sample 100 g of fruits that were equally ripe at the stage when they turned fully red, from first, second and third fruit order in 2003 and 2004. In 2005 fruit samples were collected as average of fruit orders. The fruits were frozen at −20 °C before delivered by air to the University of Kuopio, Finland for analyses. The analyses were °Brix, titratable acid and L-ascorbic acid in 2003 and 2004. In 2005 total phenolics, total anthocyanins and ellagic acid were also analysed.

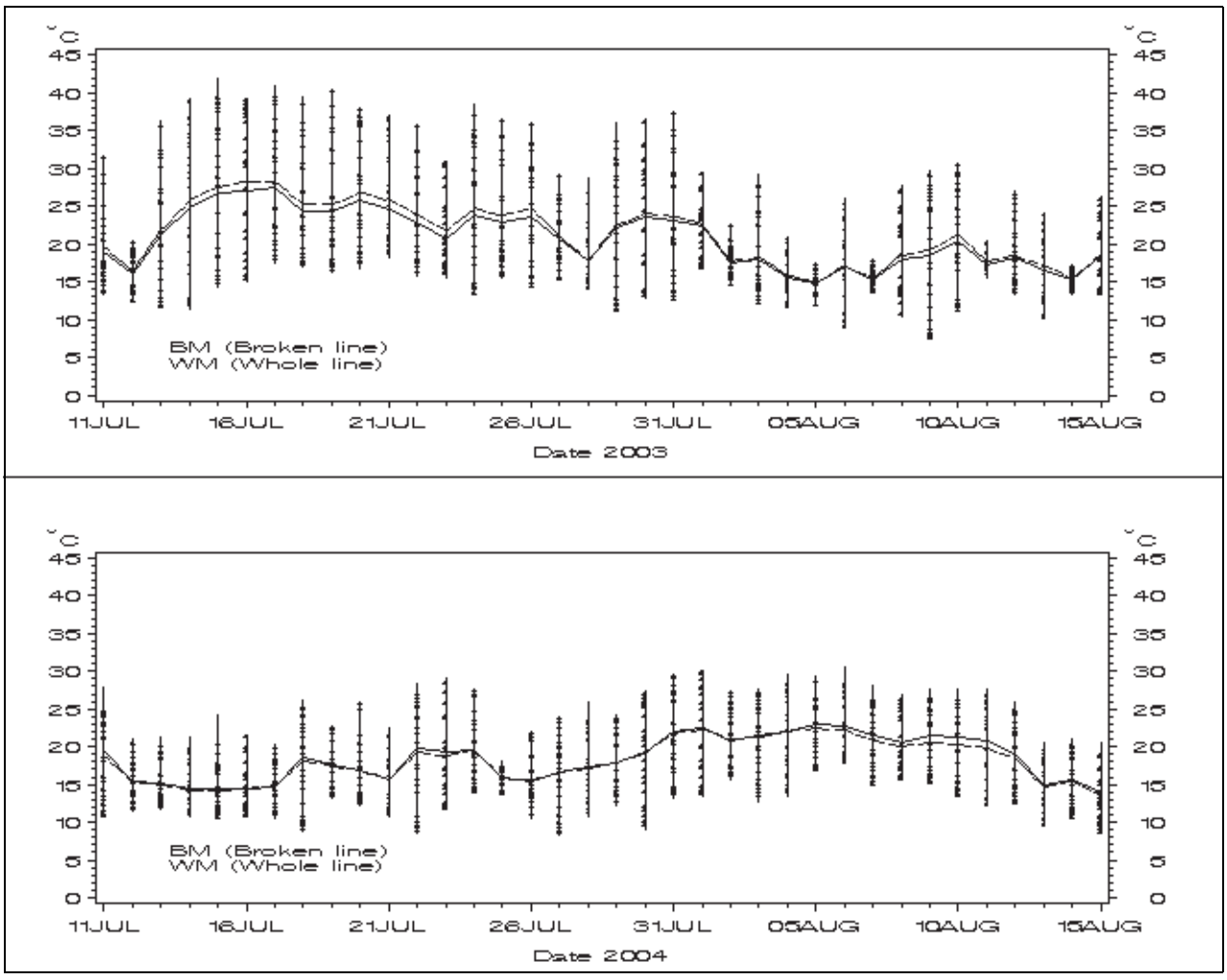


Fig. 2. Effect of white and brown polyethylene mulch, on the daily average, high and low temperatures in the leaf canopy of 'Korona' strawberry. (Observation period: 2003–2005; Site of temperature sensor: 5 cm above ground).

Statistics were performed using the SAS 'GLM', 'Graph' and 'Tabulate' procedures (SAS 2001).

Analysis of biochemical compounds

For all analyses frozen fruits were thawed in a microwave oven and homogenized using a food processor to create a slurry. The microwave oven was a generic household oven with rotating plate (UPO, Model 800, AM Kodinkoneet OY, Helsinki, Finland). Fruits were thawed for about 1 min at 650 W. In addition, berries were mixed a few times during thawing to avoid the formation of local heat points. The thawing was controlled as to keep the temperature of the fruits close to 0 °C to avoid degradation. Single analyses of each quality parameter were made on random samples from each plot of the field experiment.

°Brix values were measured of the juice samples that were separated from the slurry by centrifugation (4420 g, in 10 minutes). Measurements were taken using an Atago Digital Refractometer PR-32 (Atago Japan). Results are expressed as % of soluble solids w/w. Total acids were measured as titratable acids using the AOAC official method 942.15 (CUNNIF 1998). Juice was diluted in deionized water and titrated with 0.1 M NaOH [sodium hydroxide purchased as 50 % solution from Fluka (Buchs SG Switzerland)] to pH 8.1. Results are expressed as g citric acid 100 g^{-1} of juice.

Vitamin C was measured as L-ascorbic acid. Samples (5 g) from the slurry, still frosty, were extracted with ice-cold meta-phosphoric acid (50 ml; 1.5 % (W/v); pH 3.5) for 10 minutes, using a commercial colorimetric method based on the reduction of tetrazolium MTT (catalog no. 0409677, Boehringer Mannheim/R.Biopherm GmbH, Darmstadt, Germany). The results are expressed as mg kg^{-1} FW.

Total anthocyanins in the extracts (ANTTONEN et al. 2006) were quantified using the pH differential method (CHENG and BREEN 1991). Results are expressed as mg 100 g^{-1} FW. In calculations, the molecular weight and the molar absorptivity of pelargonidin-3-glucoside (433 g mol^{-1} and 22400 l mol^{-1} cm^{-1}, respectively) were used.

Total phenols in the extract (ANTTONEN et al. 2006) were quantified following SINGLETON and ROSSI (1965), with minor modifications. Volumes of the extract, Folin-Ciocalteu's phenol reagent and sodium carbonate were reduced to 1:10 compared to the original method with final volume of 20 ml. The modified method was found to give comparable results to the original. Gallic acid was used for quantification and results are expressed as mg 100 g^{-1} FW.

Ellagic acid was analysed from fruit extracts using HPLC based methods after acid hydrolysis as described in ANTTONEN and KARJALAINEN (2005). In strawberries, ellagic acid is found mainly as glycosides and as hydrolysable ellagitannins (MÄÄTTÄ-RIIHINEN et al. 2004). The aglycone released by the acid hydrolysis, was identified and quantified using a pure standard compound. Results are expressed in mg 100 g^{-1} FW.

Results

There were no significant interactions between combinations of treatments expressed as fruit quality compounds or yield parameters in any year. However, there were significant interactions between fruit order and year in levels of °Brix (P=0.0156) and vitamin C (P=<0.0001) (Table 1).

Effects of fruit order, mulch colour and forcing on fruit quality components

In 2003, first and third order fruits had higher levels of °Brix, total acids and vitamin C than fruits of second order. In 2004, °Brix level was close to the 2003 level, and first order fruits had higher content than second and third order fruits. Contents of vitamin C in 2004 gradually decreased by increasing fruit order, and the level was lower than in 2003, while there was no effect on total acids (Table 1).

°Brix level was not affected by mulch-type in 2003 or 2005, but was higher in 2004 on WM compared to BM. Mulch type had no effect on total acids in either year. WM more so than BM affected fruits to produce higher levels of vitamin C in all years. In 2005, ellagic acid and total phenolics were higher on WM compared to BM, while total anthocyanins was not affected by mulch reflectivity (Table 2). Generally, the level of compounds decreased throughout the years.

Forcing had no significant effects on fruit quality components in any year (Table 1)

Table 1. Quality components of fruits affected by fruit order in a late (2003) and in a mid (2004) season tunnel production of 'Korona' strawberry.

Order	2003			2004		
	°Brix (% soluble solids)	Total acids (g $100g^{-1}$)	Vitamin C (mg kg^{-1})	°Brix (% soluble solids)	Total acids (g $100g^{-1}$)	Vitamin C (mg kg^{-1})
First	10.6	0.94	319	10.7	0.83	305
Second	9.6	0.88	292	9.9	0.82	281
Third	11.2	0.95	324	9.9	0.86	247
SE[y]	0.3***	0.02***	13*	0.2**	0.02ns	11***

[y]Standard error; *,**,***, and ns, indicate P=0.05; P=0.01 and P=0.001 and no significance, respectively.

Table 2. Level of quality compounds in fruits affected by mulch colour in late (year 2003) and in mid season (years 2004 and 2005) tunnel productions of 'Korona' strawberry.

Mulch	°Brix (% soluble solids)	Total acids (g 100g^{-1})	Vitamin C (mg kg^{-1})
Year 2003			
Brown	10.4	0.91	294
White	10.5	0.93	329
SE[y]	0.2 ns	0.01 ns	10**
Year 2004			
Brown	9.9	0.84	269
White	10.5	0.84	287
SE	0.2**	0.02 ns	9*
Year 2005			
Brown	8.7	0.76	249
White	8.6	0.75	268
SE	0.2 ns	0.02 ns	8**

Mulch	Ellagic acid (mg 100g^{-1})	Total anthocyanins (mg 100g^{-1})	Total phenolics (mg kg^{-1})
Brown	41.1	50.9	276
White	44.1	52.6	291
SE	0.7*	1.5 ns	4**

[y]Standard error; *,**,***, and ns, indicate P=0.05; P=0.01 and P=0.001 and no significance, respectively.

Table 3. Correlation coefficients (r) and probabilities (P), between listed fruit quality components and total yield in a mid season tunnel production system of 'Korona' strawberry in two years.

Quality components	2004		2005	
	r	P	r	P
°Brix	−0.25	0.1636	−0.48	0.0005
Vitamin C	−0.51	0.0026	−0.44	0.0016
Total acid	−0.71	<0.0001	−0.08	0.5967
Total phenolics			−0.24	0.0989
Total anthocyanins			0.04	0.7811
Ellagic acid			−0.04	0.7636

Correlations between fruit yield and quality parameters

In 2004, fruit yields were negatively correlated with the contents of vitamin C and total acids, and they tended to be negatively correlated with °Brix level. These relations were enhanced in 2005 for °Brix and vitamin C. Total phenolics tended to be negatively correlated with yield, while there was no correlation between yield and total acids, total anthocyanins or ellagic acid in 2005 (Table 3).

Effects of mulch colour and early forcing on yield parameters and chlorophyll

Fruit yield in 2003 was below 400 kg 1000 m^{-2} independent of treatment, while yield was about four times as high in 2004 and 2005 (Table 4). Mulch colour did not affect total yield in 2003 or 2004, but BM increased the yield in 2005 compared to WM. There were more large saleable

Table 4. Effect of mulch colour on yield, fruit size and percentage of yield in different classifications, in late (year 2003) and mid seasons (years 2004 and 2005) tunnel production of 'Korona' strawberry.

Mulch	Yield (kg 1000m^{-2})	Fruit size (g fruit^{-1})	Percentage of saleable fruit (≥25 mm)	Percentage of total yield		
				Misshape	Mildew	Rot
Year 2003						
Brown	378	14.2	89.2	6.5	11.0	1.0
White	396	16.1	94.3	7.4	8.4	0.9
SE[y]	41 ns	0.5**	1.3**	1.4 ns	2.8 ns	0.3 ns
Year 2004						
Brown	1658	20.7	81.3	0.7	11.9	0.4
White	1483	20.7	86.2	1.0	7.4	0.2
SE	77 ns	0.8 ns	3.4 ns	0.3 ns	3.4 ns	0.1 ns
Year 2005						
Brown	1747	12.6	68.5	1.1	13.0	0.3
White	1462	12.6	72.0	1.0	9.2	0.5
SE	127**	0.6 ns	2.7 ns	0.2 ns	2.8 ns	0.1 ns

[y]Standard error; *,**,***, and ns, indicate P=0.05; P=0.01 and P=0.001 and no significance, respectively.

Table 5. Effect of mulch colour and forcing on relative[x] amount of chlorophyll at anthesis and at first harvest in 2003, and at anthesis in 2004 under respectively late and mid season tunnel production of 'Korona' strawberry.

	Year 2003		Year 2004
	Anthesis	First harvest	Anthesis
Mulch			
Brown	100	87	97
White	102	89	99
SE[y]	0.9ns	0.7**	0.9**
Forcing			
Control[x]	100	87	101
Forcing	104	90	97
SE[y]	0.9***	0.7***	0.9**

[x]Control at anthesis in 2003 is 100.
[y]Standard error; *,**,***, and ns, indicate P=0.05; P=0.01 and P=0.001 and no significance, respectively.

fruits using WM compared to BM in 2003 and tended to be more in 2004 and 2005. Fruit size was larger using WM compared with BM in 2003 but not in 2004 and 2005. There were no recorded effects of mulch treatments on fruit rot or misshapen fruits in either year. However, there was a tendency to more mildew on BM compared to WM, in all years. The relative amount of chlorophyll was higher using WM compared with BM at first fruit harvest (FFH) in 2003 and at anthesis in 2004 (Table 5), and the level dropped from anthesis to FFH.

Forcing increased the amount of chlorophyll in 2003 at anthesis and at first fruit harvest (FFH), but had the opposite effect at anthesis in 2004 (Table 5). Forcing in 2003 reduced the yield by 22 % and reduced fruit size and the percentage of saleable fruits of diameter ≥25 mm, and increased the percentage of misshapen fruits (Table 6). There was no recorded effect of forcing on mildew or fruit rot, but a gain in earliness of four days. In 2004 there were no recorded effects on yield parameters of forcing, except for a seven days gain in earliness, compared with no forcing.

Discussion

There were large differences in fruiting season and yield between the first and later fruiting years, which created an interaction between year and fruit order expressed in levels of BCs. The similarities between the years were that first order fruits had higher levels of °Brix, total acids and vitamin C than second order fruits. The differences were that in the first year the levels in third order fruits, rose to the same levels as in first order fruits, while in the second year they remained at the same level as in second order fruits, or continued to drop as for vitamin C. The observed drop in quality contents during secondary fruit development in 2003 could be caused by DLIs often close to or below 2.4 MJ m^{-2} d^{-1}, a level that was below light saturation (Elsacker et al. 1989; Yun and Yoo 1992), and had a negative effect on 'Korona' strawberry yield (Fletcher et al. 2002; Awang and Atherton 1995). The observed drop in fruit quality in 2004 and 2005 during secondary and third fruit development could not be caused by low DLIs, but probably by a dilution effect caused by the relatively high yield during these periods, as stated here by the negative correlation found in 2004 and 2005 between yield and °Brix and in 2004 between yield and total acids. The differences in °Brix and total acids, values important for fruit taste (Alavoine and Crochon 1989), between fruit orders do not indicate large differences in taste. It can therefore be concluded that large differences in taste of fruits on a single plant can not be caused by the observed differences. It has to be caused by other factors such as injuries on the crowns due to fungi, freezing or mechanical injury, that interfere with uptake and transport of minerals, water and assimilates.

White mulch, presumably by increasing DLIs, yielded a higher content of chlorophyll in the leaves. Under the low light regime in the tunnel this should have a potential to increase level of fruit quality parameters and yield. This theory was supported by the results, which showed

Table 6. Effect of forcing on yield, fruit size and percentage of yield in different classifications, in late (year 2003) and mid seasons (year 2004) tunnel production of 'Korona' strawberry.

Treatment	Yield (kg 1000m^{-2})	Fruit size (g $fruit^{-1}$)	Percentage of saleable fruit (≥25 mm)	Percentage of total yield		
				Misshape	Mildew	Rot
Year 2003						
Control	434	17.6	96.7	4.9	10.6	1.1
Forcing	340	12.6	86.7	9.2	8.8	0.8
SE[y]	41 ns	0.5**	1.3**	1.4**	2.8 ns	0.3 ns
Year 2004						
Control	1571	21.1	82.4	0.9	11.2	0.2
Forcing	1570	20.3	85.1	0.8	8.0	0.4
SE	77 ns	0.8 ns	3.4 ns	0.3 ns	3.4 ns	0.1 ns

[y]Standard error; *,**,***, and ns, indicate P=0.05; P=0.01 and P=0.001 and no significance, respectively.

that WM either produced fruits with higher contents of BCs for example, vitamin C in all years, °Brix in 2004 and ellagic acid and total phenolics in 2005, or kept contents at the same level as for BM for the other compounds. However, yield was reduced in two of the three years on WM compared to BM, but this was an effect of more freezing injury on WM than on BM the winter between the first and second fruiting year.

Forcing using row-cover had no clear direct effect on quality compounds, but in both years it tended to increase vitamin C levels, probably due to a high temperature stress during forcing in accordance with WANG and ZENGH (2001) and ATKINSON et al. (2005). Forcing in 2003 had a positive effect on freezing tolerance the following winter, which can be explained by production of stress proteins, which also are effective protectors of freezing injury (LARCHER 2003). To conclude, row-cover had no effect on the recorded compounds, but could cause reduction in fruit size and a higher degree of fruit malformation, so the only argument to recommend row-cover is for forcing the harvesting season.

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Addresses of authors: Rolf Nestby (corresponding author), The Norwegian Crop Research Institute, Kvithamar Research Centre, N–7500 Stjørdal, Norway, Mikko J. Anttonen and R. Karjalainen, University of Kuopio, Institute of Applied Biotechnology, Box 1627, FIN 70211 Kuopio, Finland, and M. Verheul, The Norwegian Crop Research Institute, Særheim Research Centre, N-7500 Klepp st., Norway,
e-mail: rolf.nestby@bioforsk.no.