

## Differences in Fruit Color Development, Anthocyanin Content, Yield and Quality of Seven 'Delicious' Apple Strains

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### Abstract

Seven strains of 'Delicious' apple (*Malus domestica* Borkh.) were evaluated over a three-year period in the area of Lleida (NE Spain) for fruit color, anthocyanin content, yield and fruit quality. Fruit color was measured at two locations on each fruit blush side and back side at the midpoint between the stem and calix end, with a Minolta Chroma Meter CR-200 portable tristimulus colorimeter using the CIE [1976]  $L^*a^*b^*$  system providing an objective determination of the three visual attributes of color (lightness, hue angle and saturation). Anthocyanin content of corresponding skin disks was determined spectrophotometrically. Significant differences were observed between strains in both the chromaticity values and the distribution of anthocyanin on both sides of the fruit, even at the first measurement date. Strains which had a darker red coloration tended to initiate color development earlier. In all strains, rapid color development occurred between the middle of August and the first week of September. High-coloring strains such as 'Red Miracle,' 'Red Chief' (Campspur) or 'Early Red One' had significantly higher anthocyanin content, and lower hue angle ( $\phi$ ) and  $L^*$  values on both sides of the fruit than low-coloring strains such as 'Topred' or 'Sharpred.' Fruit color was influenced by the strain and also by the weather conditions of the particular year. Whereas in 1993 low temperatures prior to harvest decreased the effects of strain on fruit color, in 1992 and 1994 average temperatures were warm, and there were more differences between strains. The temperatures during the current seasons influenced fruit color, and consequently the season factor and also its interaction with sampling dates were significant. At commercial harvest, a linear regression of anthocyanin content of all strains on the hue angle,  $L^*$  and  $a^*/b^*$  ratio, provided  $R^2$  values of 0.79, 0.80 and 0.82, respectively. 'Early Red One' and 'Sharpred' were among strains with high cumulative yields. 'Red Chief' (Campspur), 'Elite' and 'Early Red One' had relatively high firmness at commercial harvest, and 'Oregon Spur' (Trumdor) and 'Topred' the lowest. 'Sharpred' and 'Topred' had the highest soluble solids concentrations and 'Early Red One,' 'Elite' and 'Red Chief' the lowest ones. Higher titratable acidity were obtained with 'Red Miracle' and 'Topred.'

### Introduction

Red color development in the apple is influenced by cultural and environmental factors. Two environmental factors key to the development of the red pigment, light (2, 11, 29), and temperature (1, 4, 14). In addition to environmental factors, anthocyanin synthesis is also influenced by genotype; different strains present different aptitudes to produce cells capable of anthocyanin synthesis. The influence of temperature on coloring is also dependent on the cultivar (6), so each cultivar has a different temperature that was optimum for anthocyanin accumulation (11, 30). Within the same group (i.e., 'Delicious') new selected strains are highly colored,

even in the environmental conditions of hot areas that are largely unfavourable to color development. These strains with a high anthocyanin level on the side unexposed to light would produce fruit with improved color under low-light or shaded conditions.

Different aspects of fruit quality, particularly color, have been reported for 'Delicious' strains (5, 10, 22). In red apples, the quality in the market place is determined in the first instance by a striking visual appearance. The intensity and quality of red skin color influences both consumer acceptance and sales (15). Usually the lesser the intensity of the fruit's color the lower the packing grade is, and

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therefore the lower the financial return to the growers (7). This problem is particularly important in the majority of mediterranean producing areas with hot dry weather in summer unfavorable conditions for fruit color development.

In the region of Lleida (Ebro Valley, NE Spain), 'Delicious' strains are second only to the 'Golden Delicious' group in number and account for 18% of orchard acreage. Among these varieties the most important is 'Starking Delicious' which in recent years has exhibited a substantial decrease in acreage due to its poor coloration, especially in years of high temperatures and stress conditions during the preharvest period. The achievement of a red blush over much of the surface is of particular importance, but this blush development often occurs at or past optimum harvest maturity. For this reason harvest is usually delayed in order to attain a certain degree of color, affecting negatively fruit firmness and flavour (13). In commercial orchards in the region, a range of techniques to improve color have been used; application of growth regulators, and evaporative cooling by sprinkler irrigation. Recently, the loss of Daminozide to delay maturity and increase red color and firmness, mean that evaporative cooling is now one of the few possibilities that growers have for improving color. However, in new orchards the most effective way to combat the problems of poor coloration is the introduction of new strains such as 'Red Chief' or 'Early Red One' which have high coloring potential, even under the environmental conditions of warm areas (16, 18, 19, 35).

Analytical determination of anthocyanin content has been widely utilised in the search for apple color, because higher anthocyanin content is associated with higher fruit color (8, 16, 31). This procedure involves extracting anthocyanin from the fruit skin and measuring it spectrophotometrically, and is thus both time consuming and destructive. In contrast, the development of portable, easy-to-operate, tristimulus colorimeters permit the rapid, subjective and nondestructive

quantification of fruit color and the estimation of fruit anthocyanin levels in internationally acceptable units (31, 33).

This study was conducted over 1992-1994 growing seasons, and skin color development, anthocyanin content, yield and fruit quality of seven 'Delicious' apple strains in the Lleida region were studied. The main objectives were to determine the influence of strain on fruit color development and fruit quality, and to correlate anthocyanin content to fruit chromaticity values measured instrumentally.

## Materials and Methods

### *Plant material, experimental design, sample collection*

Fruit were obtained from the 'Delicious' apple strain evaluation block at the Seana Experimental Orchard (Lleida, Spain). 'Red Chief' (Campspur), 'Early Red One' and 'Red Miracle' (categorized as standard but bearing habit similar to spur), 'Elite' and 'Oregon Spur' (Trum-dor), were grafted on MM.106, 'Topred' and 'Sharpred' (more vigorous strains) on M.9 EMLA. The trees were trained in a modified central leader by pruning during the vegetative and dormant seasons. Trees spaced 4 x 1.5 m were planted in 1984 in north-south oriented rows on Xerocrept Petrocalcic, loam, mixed, mesic, shallow soil that averages 0.65 m depth. Recommended herbicide and pesticide programmes were followed. The fruit were not chemically thinned and hand thinning was realised in late May, to spacing fruit 7 cm each. The trees were irrigated by flooding irrigation system, with an interval irrigation of 17 to 22 days.

The experimental design was a completely randomized block design with five one-tree replications of each of the 7 strains. Full bloom dates for the strains were 15 April 1992, 14 April 1993 and 1 April 1994. The commercial harvest was performed 140 days after full bloom; as in other research projects (5, 32), fruits from all strains were harvested on the same date (commercial harvest time) each year, and a combination of several maturity attributes was considered.

Fruit samples were collected during the 4 to 6 weeks prior to the commercial harvest date, and on the commercial harvest date at four successive stages of maturity in 1992 and 1994 (from the end of July to the beginning of September) and at three stages in 1993 (early August-early September). Samples for color evaluation on the dates previous to the commercial harvest consisted of 10 fruits randomly harvested at a height of 1 to 2 m from each of five replicate trees (50 fruits/strain). On the commercial harvest date the samples consisted of 15 fruits/tree (75 fruits/strain); complementarily subsamples of 15 fruits from each five replicate trees (75 fruits/strain) were assessed for average fruit size, flesh firmness, soluble solids concentrations (SSC) and titratable acidity (TA).

#### *Fruit color measurement*

Apple color was measured with a Minolta CR-200 Chroma Meter portable tristimulus colorimeter (Minolta Co; Osaka, Japan) and fruit chromaticity was recorded in Commission Internationale d'Eclairage (C.I.E.)  $L^*$ ,  $a^*$  and  $b^*$  color space coordinates (17, 31, 32). The colorimeter was calibrated with a white standard (Minolta calibration plate CR.A43) before use. In this system of color representation the values  $L^*$ ,  $a^*$  and  $b^*$  describe a uniform three-dimensional color space, where the  $L^*$  value corresponds to a dark-bright scale (0, black; 100, white) and represents the relative lightness of colors with a range from 0 to 100, being small for dark colors and large for light colors. Both  $a^*$  and  $b^*$  scales extend from -60 to 60;  $a^*$  is negative for green and positive for red, while  $b^*$  is negative for blue and positive for yellow. Also defined in this color system are the hue angle angle ( $\emptyset$ ), calculated as  $\tan^{-1} b^*/a^* \times 57.3$  (expressed in degrees) (25).

Immediately after harvest, fruits were gently wiped with a soft cloth to remove any spray residues or dust. For each strain and sampling time, color was measured in each fruit at two opposite sides: the blush side exposed to sunlight (BL), and the

shaded or back side (BA). Color measurements were made by placing the 8mm diameter measuring area of the colorimeter at the midpoint between the stem and the calix end on both fruit sides.

#### *Anthocyanin content*

Anthocyanin content was determined immediately after color measurements at the same locations per fruit where chromaticity values were recorded. Anthocyanins were extracted from skin by placing two skin disks of 11 mm in diameter corresponding to each location of color measurement in 10 ml of a solution of 50 methanol (26.4 M): 1 HCl (35%): 49 water. Extraction was carried out overnight at 4°C in the dark. Absorbance of extracts was measured with a Cecil series 1000 spectrophotometer (Cecil Instruments, Cambridge, England) at 532 nm and the anthocyanin concentration was determined using a molar extinction coefficient of  $3.43 \times 10^4$ ; data were expressed in nmol.cm<sup>-2</sup>.

#### *Fruit quality parameters*

Flesh firmness was determined with an Effegi portable penetrometer (Effegi, Alfoncina, Italy) with an 11 mm tip, using two readings from two opposite peeled sides of 75 fruit for each strain; data were measured in kilograms and then converted to newtons (N). Soluble solids concentrations (SSC) was determined on a blended composite of wedges from 15 unpeeled apples/tree using an Atago-Palette 100 digital calibrated refractometer (Atago Co., Tokyo, Japan); data were expressed in %. Titratable acidity (TA) was determined for the same composite by titrating to an end point pH 8.2 with 0.1 N NaOH and expressed as g malic acid. L<sup>-1</sup>.

#### *Data analysis*

Data of chromaticity values, anthocyanin content and fruit quality were subjected to analysis of variance with SAS software; statistical significance was judged at  $P = 0.05$ . When the analysis was statistically significant (F-test), Duncan's new multiple range test for separa-

tion of means for sampling dates and years was performed. Differences between strains were evaluated by a factorial model of analysis of variance (with strains, trees, fruits, fruit sides and replications) by using a General Linear Model (GLM) of the Statistical Analysis System procedure (28) as a randomized complete block design to determine the statistical significance of single, two and three-way interactions. The original fruit color (chromaticity values and anthocyanin contents) and fruit quality parameters are directly reported because their distribution was normal. Regression analysis was used to correlate anthocyanin content with chromaticity values ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $a^*/b^*$ , hue angle and chroma), whose relationship was examined using different linear regression models. Coefficients of determination ( $R^2$ ) and the significance of regression models between anthocyanin contents and chromaticity values were calculated. Table Curve 2D (21) was used to adjust mathematical functions to the relationship between these two variables.

## Results and Discussion

### Chromaticity values

The mean values of  $L^*$ ,  $a^*/b^*$  (data not exposed) and hue angle corresponding to the blush side (BS), back side (BA), and means of both sides (M) for the sampled period of 1992, 1993 and 1994 seasons are shown in Table 1.

In 1992, at commercial harvest, 'Red Miracle' fruits showed lower values of hue angle (M) than 'Topred,' indicating a higher coloration; the lowest  $L^*$  value was thus obtained by 'Red Miracle' and the highest by 'Topred' (Table 1). The values obtained by the other strains were intermediate; 'Early Red One,' 'Red Chief' and 'Elite' showed similar values to 'Red Miracle' and 'Sharpred' to 'Topred.' In 1992 the three sampling dates previous to commercial harvest (28 July, 10 August and 24 August), there were also differences between strains. These data showed that apple color increased progressively; even at early stages there were significant differences

between strains. Strains with chromaticity values indicating higher color at commercial harvest also showed high color at early stages; 'Red Miracle,' 'Early Red One,' 'Red Chief' and 'Oregon Spur' differed from 'Topred.'

In 1993, the results were similar to those of 1992 (Table 1), although all chromaticity values indicated a significantly higher coloration than 1992 and 1994. Based on  $L^*$  and hue angle values obtained at commercial harvest and preharvest dates, the highest coloration was obtained for 'Red Miracle' and the lowest for 'Topred,' with intermediate values for the other strains. In 1994, at commercial harvest there were more differences between strains than on the preharvest dates (6 August, 19 August, 31 August). As in the 1992 and 1993 seasons, the lowest values for hue angle and  $L^*$  were obtained for 'Red Miracle'; intermediate and largely similar values were obtained for 'Red Chief,' 'Early Red One' and 'Oregon Spur.' No significant differences were found between 'Elite' and 'Sharpred.' As in 1992 and 1993, on the first and second measurement dates (6 August, 19 August) there were significant differences between strains, similar to those reported for the commercial harvest.

As regards the BL side, the lowest  $L^*$  values (Table 1) were obtained (on all harvest dates and season) for 'Red Miracle,' and the highest for 'Topred,' 'Sharpred' and 'Elite,' intermediate values were found for 'Red Chief,' 'Oregon Spur' and 'Early Red One.' Similar results were obtained for the BA side. Comparing the three seasons, 1992 and 1994 values were similar and higher in general than those of 1993, which indicated less fruit colour. All strains increased red color rapidly between the second half of August and the first week of September (commercial harvest date). The quantified differences in fruit red color development are in general in agreement with visual observations and the anthocyanin content of these strains.

This results are in accordance with those exposed by Baugher et al. (5) using a panel color rating; 'Red Chief,' 'Oregon

'Spur' and 'Sharpred' had most desirable color than 'Topred.' Fallahi et al. (13) reported that the color rating of 'Early Red One' was significantly higher than all other strains, while 'Oregon Spur' and 'Sharpred' had intermediate values, higher than 'Topred.' Is noteworthy that strains with high red color ('Red Chief,' 'Oregon Spur') and that with poorest red color ('Topred,' 'Sharpred') are both striped type and therefore is not possible to draw a line between stripe vs. blush strains for fruit color.

The uniformity of color on the fruit can be evaluated by the determination of colorimetric values (i.e., hue angle) separately on both sides (BL and BA) of each sampled fruit (Table 1), as reported previously by several researchers (16, 30). In the three seasons, 'Red Miracle' was the strain that presented the lowest differences between sides; its high color was associated with uniform distribution on the fruit, and an earlier development (33). For 'Red Chief,' 'Early Red One' and 'Oregon Spur' BL side values were about twice times higher than those for the BA side (in 1994); their color development was not as early as 'Red Miracle.' In contrast, lower color at commercial harvest was obtained on the strains which develop red color later, such as 'Sharpred' and 'Topred'; for these poorly colored strains there were also few differences between sides. An early development of color was related to high color at commercial harvest ('Red Miracle,' 'Red Chief' and 'Early Red One'). Similar results have been reported by other researchers with 'Red Delicious' strains (11, 30, 31), which obtained higher color on both sides of the fruit for well colored strains than for less colored ones.

#### *Anthocyanin content*

The anthocyanin content increased continuously during fruit maturation, especially in the two weeks (Table 2) preceding the commercial harvest date. As with chromaticity values, on the measurement dates prior to commercial harvest of the three seasons, significant differences were

observed between strains. At the first measurement date in the three seasons 'Red Miracle' showed generally far more anthocyanin content than the other strains. At early stages of fruit development, other researchers (5, 6, 7, 33) reported lower color of 'Topred' and 'Sharpred' than 'Red Chief,' 'Early Red One,' 'Scarlet Spur,' 'Oregon Spur' and 'Ace.' There were also marked differences between seasons, especially at commercial harvest; differences between strains were similar to those reported for chromaticity values.

In 1992 the highest anthocyanin content, measured both on the BL side and in the fruit as a whole, was obtained for 'Red Miracle.' 'Early Red One' and 'Red Chief' were second, with higher values than 'Elite' and 'Oregon Spur'; 'Topred' and 'Sharpred' had the lowest content. On the first date (28 July) there were differences that indicated no variations in color development; thus 'Red Miracle,' 'Red Chief' and 'Early Red One' had higher anthocyanin content, and there were no differences as regards the rest of strains, which showed significantly lower values on the BA side and a lack of color at early stages of fruit development (33). In 1993, the lower temperatures in the preharvest period led to higher anthocyanin content than in 1992 and 1994. As in 1992, at commercial harvest 'Red Miracle' had the highest anthocyanin content, and there were no differences on the BL side of 'Early Red One,' 'Red Chief' and 'Oregon Spur.' 'Early Red One' and 'Oregon Spur' values on the BA side were similar and higher than 'Red Chief' and 'Elite.' The poorly colored strains ('Sharpred' and 'Topred') had the lowest content on both fruit sides. In 1994, the temperatures were high and the anthocyanin content were lower than in 1993 and similar to 1992; the results were like to those of 1992 ('Red Miracle' showing the highest anthocyanin content and 'Topred' the lowest).

These results indicate that there are differences between strains in both earliness and levels of red color measured by chromaticity values and anthocyanin con-

**Table 1. Mean colorimetric values\* ( $L^*$  and hue angle) during the period preceding the commercial harvest and at commercial harvest ( $\Phi$ ), corresponding to the mean of both fruit sides (M), blush side (BL) back side (BA) in seven 'Delicious' strains in the 1992, 1993 and 1994 seasons.**

1992		28 July			10 August			24 August			7 September $\Phi$		
Parameter	Strain	M	BL	BA	M	BL	BA	M	BL	BA	M	BL	BA
$L^*$	Red Miracle	44.6 <sup>c</sup>	40.4 <sup>cd</sup>	48.8 <sup>ce</sup>	44.9 <sup>a</sup>	41.2 <sup>ce</sup>	48.6 <sup>cd</sup>	34.3 <sup>e</sup>	31.9 <sup>ce</sup>	36.7 <sup>ce</sup>	31.2 <sup>f</sup>	30.1 <sup>cd</sup>	32.2 <sup>ce</sup>
	E. Red One	62.2 <sup>b</sup>	56.1 <sup>bc</sup>	68.4 <sup>ab</sup>	57.2 <sup>bc</sup>	50.8 <sup>bcd</sup>	63.6 <sup>bc</sup>	47.2 <sup>d</sup>	42.2 <sup>d</sup>	52.3 <sup>b</sup>	39.3 <sup>de</sup>	34.7 <sup>c</sup>	41.1 <sup>d</sup>
	Red Chief	61.6 <sup>b</sup>	55.0 <sup>c</sup>	68.3 <sup>ab</sup>	57.4 <sup>b</sup>	52.1 <sup>bcd</sup>	62.8 <sup>bc</sup>	48.1 <sup>d</sup>	42.7 <sup>d</sup>	53.6 <sup>b</sup>	41.0 <sup>cde</sup>	37.9 <sup>c</sup>	44.0 <sup>cd</sup>
	Elite	62.9 <sup>b</sup>	57.8 <sup>bc</sup>	68.0 <sup>b</sup>	53.8 <sup>c</sup>	48.3 <sup>d</sup>	59.3 <sup>c</sup>	48.2 <sup>d</sup>	43.9 <sup>cd</sup>	52.5 <sup>b</sup>	38.4 <sup>e</sup>	36.5 <sup>c</sup>	40.4 <sup>d</sup>
	Oregon Spur	63.5 <sup>b</sup>	58.0 <sup>bc</sup>	69.0 <sup>ab</sup>	59.4 <sup>b</sup>	53.0 <sup>bc</sup>	65.9 <sup>ab</sup>	51.0 <sup>cd</sup>	45.6 <sup>c</sup>	56.4 <sup>ab</sup>	42.1 <sup>cd</sup>	39.5 <sup>c</sup>	44.6 <sup>bcd</sup>
	Sharpred	62.9 <sup>b</sup>	57.7 <sup>bc</sup>	68.2 <sup>b</sup>	54.2 <sup>c</sup>	49.2 <sup>cd</sup>	59.3 <sup>c</sup>	56.3 <sup>ab</sup>	50.6 <sup>ab</sup>	61.9 <sup>a</sup>	45.9 <sup>ab</sup>	43.3 <sup>ab</sup>	48.5 <sup>ab</sup>
	Topred	68.2 <sup>a</sup>	65.4 <sup>a</sup>	71.1 <sup>a</sup>	64.3 <sup>a</sup>	59.3 <sup>a</sup>	69.4 <sup>a</sup>	57.3 <sup>a</sup>	52.3 <sup>a</sup>	62.3 <sup>a</sup>	48.3 <sup>a</sup>	44.7 <sup>a</sup>	52.0 <sup>a</sup>
Hue angle (°)	Red Miracle	56.9 <sup>c</sup>	61.3 <sup>d</sup>	52.5 <sup>b</sup>	42.2 <sup>c</sup>	29.0 <sup>c</sup>	55.4 <sup>c</sup>	21.9 <sup>f</sup>	21.7 <sup>e</sup>	22.2 <sup>e</sup>	17.8 <sup>d</sup>	17.7 <sup>c</sup>	17.9 <sup>e</sup>
	E. Red One	90.7 <sup>ab</sup>	75.7 <sup>bc</sup>	107.7 <sup>a</sup>	72.6 <sup>ab</sup>	51.0 <sup>b</sup>	94.2 <sup>ab</sup>	44.8 <sup>de</sup>	32.1 <sup>cd</sup>	57.6 <sup>cd</sup>	24.7 <sup>c</sup>	21.6 <sup>bc</sup>	27.8 <sup>d</sup>
	Red Chief	82.9 <sup>b</sup>	61.2 <sup>c</sup>	104.5 <sup>a</sup>	64.3 <sup>b</sup>	44.4 <sup>bc</sup>	84.2 <sup>b</sup>	40.6 <sup>e</sup>	28.0 <sup>de</sup>	53.2 <sup>d</sup>	25.3 <sup>c</sup>	21.5 <sup>bc</sup>	29.2 <sup>d</sup>
	Elite	83.9 <sup>ab</sup>	78.1 <sup>bc</sup>	109.7 <sup>a</sup>	67.2 <sup>b</sup>	47.3 <sup>b</sup>	87.1 <sup>ab</sup>	52.3 <sup>bcd</sup>	38.7 <sup>bc</sup>	65.9 <sup>bcd</sup>	28.8 <sup>bc</sup>	24.2 <sup>b</sup>	33.5 <sup>bcd</sup>
	Oregon Spur	95.3 <sup>ab</sup>	78.8 <sup>abc</sup>	111.8 <sup>a</sup>	76.8 <sup>ab</sup>	54.0 <sup>b</sup>	99.5 <sup>ab</sup>	50.4 <sup>cde</sup>	34.3 <sup>cd</sup>	66.5 <sup>abcd</sup>	27.9 <sup>bc</sup>	24.5 <sup>b</sup>	31.3 <sup>cd</sup>
	Sharpred	93.9 <sup>ab</sup>	78.1 <sup>bc</sup>	109.7 <sup>a</sup>	68.7 <sup>b</sup>	50.3 <sup>b</sup>	87.1 <sup>ab</sup>	62.4 <sup>ab</sup>	44.0 <sup>b</sup>	80.7 <sup>ab</sup>	32.0 <sup>b</sup>	26.5 <sup>b</sup>	37.5 <sup>bc</sup>
	Topred	105.8 <sup>a</sup>	99.4 <sup>a</sup>	112.2 <sup>a</sup>	89.1 <sup>a</sup>	71.4 <sup>a</sup>	106.8 <sup>a</sup>	69.3 <sup>a</sup>	54.1 <sup>a</sup>	84.5 <sup>a</sup>	41.6 <sup>a</sup>	32.7 <sup>a</sup>	50.5 <sup>a</sup>
1993		11 August			25 August			10 September $\Phi$					
$L^*$	Red Miracle	—	—	—	39.7 <sup>d</sup>	36.1 <sup>e</sup>	43.2 <sup>e</sup>	38.1 <sup>e</sup>	35.0 <sup>d</sup>	41.1 <sup>e</sup>	31.5 <sup>d</sup>	30.5 <sup>d</sup>	32.6 <sup>d</sup>
	E. Red One	—	—	—	50.5 <sup>c</sup>	45.0 <sup>d</sup>	56.0 <sup>d</sup>	48.0 <sup>d</sup>	44.7 <sup>c</sup>	51.4 <sup>d</sup>	38.2 <sup>c</sup>	36.2 <sup>c</sup>	40.1 <sup>c</sup>
	Red Chief	—	—	—	50.8 <sup>c</sup>	45.3 <sup>d</sup>	56.3 <sup>d</sup>	49.0 <sup>cd</sup>	44.6 <sup>c</sup>	53.4 <sup>d</sup>	39.2 <sup>bc</sup>	36.5 <sup>c</sup>	41.9 <sup>bc</sup>
	Elite	—	—	—	55.7 <sup>b</sup>	48.5 <sup>bc</sup>	63.0 <sup>ab</sup>	51.4 <sup>c</sup>	45.8 <sup>c</sup>	56.9 <sup>bc</sup>	38.9 <sup>bc</sup>	35.4 <sup>c</sup>	42.5 <sup>bc</sup>
	Oregon Spur	—	—	—	52.3 <sup>c</sup>	46.5 <sup>cd</sup>	58.0 <sup>cd</sup>	50.6 <sup>cd</sup>	46.2 <sup>c</sup>	55.0 <sup>cd</sup>	39.0 <sup>c</sup>	36.4 <sup>c</sup>	41.6 <sup>c</sup>
	Sharpred	—	—	—	56.0 <sup>b</sup>	50.9 <sup>b</sup>	61.2 <sup>bc</sup>	54.5 <sup>b</sup>	49.6 <sup>b</sup>	59.4 <sup>ab</sup>	41.6 <sup>b</sup>	39.1 <sup>b</sup>	44.0 <sup>b</sup>
	Topred	—	—	—	60.1 <sup>a</sup>	55.7 <sup>a</sup>	64.6 <sup>a</sup>	57.3 <sup>a</sup>	53.6 <sup>a</sup>	61.1 <sup>a</sup>	45.8 <sup>a</sup>	43.9 <sup>a</sup>	47.8 <sup>a</sup>
Hue angle (°)	Red Miracle	—	—	—	25.8 <sup>e</sup>	21.1 <sup>d</sup>	30.5 <sup>e</sup>	23.7 <sup>d</sup>	19.9 <sup>c</sup>	27.5 <sup>d</sup>	18.2 <sup>c</sup>	17.3 <sup>d</sup>	19.0 <sup>c</sup>
	E. Red One	—	—	—	59.4 <sup>bcd</sup>	40.4 <sup>bc</sup>	78.4 <sup>cd</sup>	47.7 <sup>c</sup>	36.6 <sup>b</sup>	57.5 <sup>c</sup>	25.1 <sup>b</sup>	21.4 <sup>bc</sup>	28.9 <sup>b</sup>
	Red Chief	—	—	—	52.9 <sup>d</sup>	35.0 <sup>c</sup>	70.8 <sup>d</sup>	45.6 <sup>c</sup>	33.3 <sup>b</sup>	57.9 <sup>c</sup>	24.1 <sup>b</sup>	20.0 <sup>c</sup>	28.3 <sup>b</sup>
	Elite	—	—	—	68.1 <sup>b</sup>	41.2 <sup>bc</sup>	95.0 <sup>ab</sup>	54.6 <sup>bc</sup>	37.0 <sup>b</sup>	72.3 <sup>b</sup>	27.9 <sup>b</sup>	21.8 <sup>bc</sup>	33.9 <sup>b</sup>
	Oregon Spur	—	—	—	56.9 <sup>cd</sup>	35.9 <sup>c</sup>	77.8 <sup>cd</sup>	45.7 <sup>c</sup>	35.9 <sup>b</sup>	55.6 <sup>c</sup>	24.6 <sup>bc</sup>	22.9 <sup>bc</sup>	26.3 <sup>bc</sup>
	Sharpred	—	—	—	65.8 <sup>bc</sup>	46.4 <sup>b</sup>	85.2 <sup>bc</sup>	58.1 <sup>b</sup>	41.3 <sup>b</sup>	74.9 <sup>b</sup>	28.2 <sup>b</sup>	24.3 <sup>b</sup>	32.2 <sup>b</sup>
	Topred	—	—	—	85.0 <sup>a</sup>	68.0 <sup>a</sup>	102.1 <sup>a</sup>	75.1 <sup>a</sup>	60.9 <sup>a</sup>	89.3 <sup>a</sup>	39.7 <sup>a</sup>	32.9 <sup>a</sup>	46.6 <sup>a</sup>

Table 1. (Continued).

1994 Parameter	Strain	6 August			19 August			31 August			6 September		
		M	BL	BA	M	BL	BA	M	BL	BA	M	BL	BA
L*	Red Miracle	50.1 <sup>+</sup> d	41.1 <sup>++</sup> e	59.1 <sup>++</sup> c	42.3 <sup>+</sup> d	47.9 <sup>++</sup> c	36.7 <sup>++</sup> c	38.0 <sup>+</sup> d	33.8 <sup>++</sup> d	42.2 <sup>+</sup> d	37.5 <sup>+</sup> d	33.3 <sup>++</sup> e	42.0 <sup>++</sup> d
	E. Red One	61.7 c	53.6 cd	69.8 ab	54.9 c	46.7 c	63.2 b	48.7 c	41.7 c	55.7 c	45.7 c	37.5 d	54.0 c
	Red Chief	61.1 c	52.3 d	69.9 ab	53.9 c	44.6 c	63.3 b	49.3 bc	41.2 c	57.4 c	46.9 c	39.1 cd	54.8 c
	Elite	62.8 bc	55.1 bc	70.6 a	55.3 c	48.6 bc	61.9 b	55.0 a	46.7 ab	63.2 ab	50.2 b	40.7 c	59.7 ab
	Oregon Spur	61.1 c	54.2 cd	67.9 b	54.0 c	45.7 c	62.4 b	49.7 bc	42.3 bc	56.9 c	46.2 c	38.6 d	53.8 c
	Sharpred	64.2 ab	57.3 b	71.2 a	54.5 bc	44.9 abc	64.2 b	53.6 ab	46.9 ab	60.4 bc	51.5 b	44.6 b	58.4 b
	Topred	66.0 a	61.0 a	71.0 a	61.2 a	55.3 a	67.2 a	55.5 a	47.6 a	63.5 ab	55.5 a	48.8 a	62.2 a
Hue angle (°)	Red Miracle	54.6 f	24.5 d	84.8 b	33.0 c	46.5 ab	19.6 c	18.8 c	13.2 e	24.4 c	21.6 f	16.8 f	26.3 f
	E. Red One	86.0 cde	60.8 b	111.2 a	64.2 b	36.8 b	91.6 b	47.4 b	27.9 cd	66.8 b	41.2 de	24.5 cde	57.9 de
	Red Chief	79.1 e	49.3 c	108.8 a	58.0 b	30.7 b	86.2 b	45.9 b	26.4 d	65.5 b	35.6 e	21.0 ef	50.3 de
	Elite	88.7 bc	63.8 b	113.6 a	65.5 b	41.8 ab	89.2 b	65.5 a	37.7 ab	92.0 a	49.6 bc	28.5 bcd	70.7 bc
	Oregon Spur	80.3 de	55.1 bc	105.5 a	58.9 b	32.8 b	85.0 b	45.2 b	25.7 d	64.7 b	35.9 e	22.9 de	48.9 e
	Sharpred	86.5 cd	63.7 b	109.3 a	63.1 b	41.2 ab	84.9 b	53.4 b	34.5 bc	72.4 b	45.2 cd	29.5 bc	61.0 cd
	Topred	96.0 a	79.3 a	112.8 a	80.5 a	56.7 a	104.3 a	68.3 a	39.5 ab	91.7 a	60.7 a	39.7 a	81.8 a

\*Mean separation within cultivars in the same row for the same year and date by Duncan's new multiple range test,  $P = 0.05$ , if the F-test was significant in the ANOVA. For symbols (+, ++, +++) see Table 2. Season factor was significant ( $P \leq 0.05$ ) at commercial harvest, for L\* and hue angle. Season x sampling date interaction was also significant ( $P < 0.01$ ) for L\* and hue angle.

tent. As suggested by Singha et al. (33), strains that are highly colored at commercial harvest (such as 'Oregon Spur', 'Ace', 'Early Red One' and 'Red Chief') develop red color at early stages and the differences were maintained through maturation. The L\*, hue angle and anthocyanin content obtained in the three seasons made it possible to separate the strains into three groups (from high to poor red color) at commercial harvest: 1. 'Red Miracle', 'Early Red One' and 'Red Chief'; 2. 'Oregon Spur' and 'Elite'; 3. 'Sharpred' and 'Topred'. The latter group

of strains showed the least less intense red coloration.

In all strains and seasons, rapid color development occurred between the middle of August to commercial harvest (6-10 September), when the strains tend to approach their ultimate color (Tables 2 and 3). This finding is in agreement with reports of other authors (3, 23, 29, 33) indicating that the potential to accumulate anthocyanins occurs between the middle to the end of the growing season in many varieties, and that color development depends on fruit maturity (11). Generally,

anthocyanin content (especially on blushed sides) fluctuates at low values during much of the growth period and then, near maturation increases markedly (27, 33). Curry (11) suggested that, at the optimum temperature, the amount of anthocyanin accumulation in 'Scarlet Spur' was about three times that in 'Oregon Spur', and this continued even at higher temperatures, which may explain why highly colored strains (in our study 'Red Miracle') begin developing their color in the middle of the summer (July) in the hottest part of the growing season.

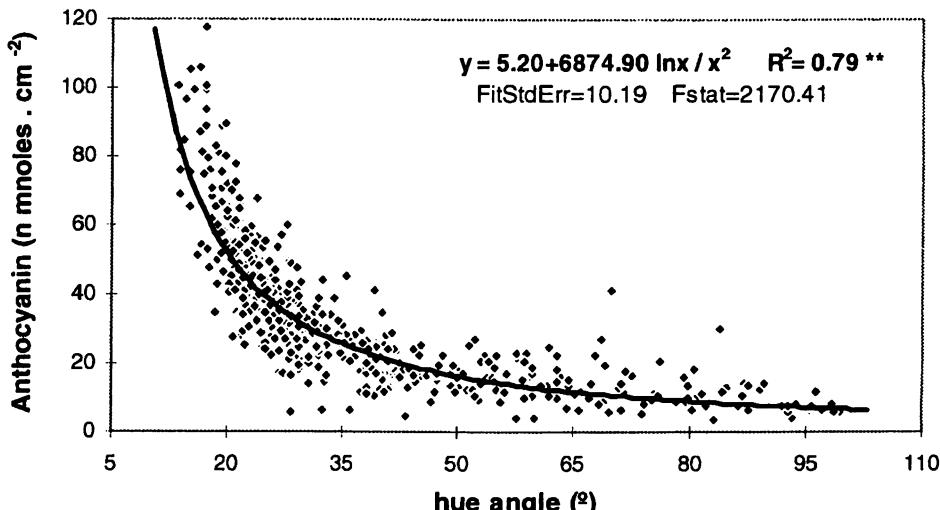


Figure 1. Relationship between extractable anthocyanin content and hue angle values measured at commercial harvest in fruit of 7 'Delicious' apple strains. Values of two seasons (1993 and 1994) are represented. Intervals of confidence at 95%: [5.20  $\pm$  1.50]; [6874.90  $\pm$  289.66].

In the three seasons, the highest anthocyanin content was obtained in 1993 (with cooler and moister weather conditions), and the lowest in 1994; inversely results were reported with the L\* and hue angle (Table 1, Table 2). The fact that color was higher in the 1993 than in 1992 and 1994 may be due to the beneficial effect of lower temperatures in 1993 during the 24 August-7 September period, in which the differences between 1993 and 1994 on the average of maximum and minimum temperatures were 6.3°C and 5.6°C, respectively. Similar results were obtained when 1993 and 1992 were compared (data not shown). As suggested by Curry (11), a cold period prior to harvest stimulates anthocyanin production and red color development; fruit color variations from year to year can usually be attributed to seasonal environmental differences in both the timing and duration of stress periods. Thus, it seems clear that there are large differences in seasonal potential for anthocyanin synthesis, although the time course of anthocyanin accumulation is qualitatively similar from year to year (8). These data support the findings of other authors (14,

29) concerning the positive influence of low temperatures on anthocyanin synthesis and fruit color development in 'Red Delicious' apples. In general, night temperatures below 18°C and daily moderate temperatures (20-25°C) promote red color development, but warm daily temperatures can negate the positive effect of the cold night temperatures (1), because photosynthesis occurs at a low rate and the carbohydrates needed for pigment synthesis are lacking. In addition high night temperatures would be inhibitory to anthocyanin accumulation because of increased inactivation of PAL (14, 34).

Although the influence of light and temperature is critically important in color development (26, 29), this is also dependent on strain (6, 11, 33). At commercial harvest, in 1994, anthocyanin content on the BL side was about three or five times that on the back side in low or medium-high coloring strains, and quite similar in 'Red Miracle' (Table 2). Highly colored strains develop red color on both fruit sides and anthocyanin synthesis is scarcely dependent on light exposure in comparison to poorly colored ones. As in our

**Table 2. Mean values of anthocyanin content<sup>y</sup> (nmoles.cm<sup>-2</sup>) during the period preceding the commercial harvest and at commercial harvest (Φ), corresponding to the mean of both fruit sides (M), blush side (BL) and back side (BA) in seven 'Delicious' strains in the period 1992 to 1994.**

Year/Strain	28 July			10 August			24 August			7 September Φ		
	M	BL	BA	M	BL	BA	M	BL	BA	M	BL	BA
1992												
R. Miracle	8.0 <sup>a</sup>	11 <sup>++</sup> <sup>a</sup>	5.0 <sup>++</sup> <sup>a</sup>	17 <sup>+</sup> <sup>a</sup>	21 <sup>++</sup> <sup>a</sup>	13 <sup>++</sup> <sup>a</sup>	32 <sup>+</sup> <sup>a</sup>	36 <sup>++</sup> <sup>a</sup>	28 <sup>++</sup> <sup>a</sup>	52 <sup>a</sup>	63 <sup>~a</sup>	40 <sup>~a</sup>
E. Red One	6.7 a	10.2 b	3.3 b	6.7 b	10.2 b	3.1 b	12.1 b	18.1 b	6.1 b	31.4 b	42.8 b	19.9 b
Red Chief	6.8 a	9.2 ab	4.4 ab	5.7 bc	9.7 bc	1.7 bc	11.7 b	16.1 b	7.3 b	26.5 bc	35.8 b	17.2 bc
Elite	0.50 b	0.9 c	0.2 c	4.7 bc	8.5 bc	1.0 c	10.6 bc	15.7 b	5.6 b	22.3 cd	24.9 c	19.6 b
Oregon S.	1.8 b	3.6 bc	0.01 c	3.9 cd	6.2 c	1.5 bc	7.7 bcd	8.1 c	7.3 b	19.7 cde	26.0 c	13.3 bcd
Sharpred	0.80 b	1.6 c	0.01 c	4.3 c	6.1 c	2.4 bc	5.8 cd	9.1 c	2.6 b	16.5 def	22.3 cd	10.6 cd
Topred	0.05 b	0.1 c	0.01 c	1.6 d	2.4 d	0.8 c	3.9 d	5.9 c	1.8 b	11.3 f	14.9 d	7.8 d
1993				11 August			25 August			10 September Φ		
R. Miracle	—	—	—	31.1 a	38.3 a	24.0 a	38.6 a	52.2 a	25.1 a	76.2 a	89.3 a	63.2 a
E. Red One	—	—	—	16.6 b	23.3 b	9.9 b	14.9 b	19.4 b	10.5 b	42.8 b	50.6 b	35.1 b
RedChief	—	—	—	12.4 c	17.2 c	7.7 c	13.9 bc	17.8 b	10.1 b	39.3 b	53.3 b	25.3 c
Elite	—	—	—	10.7 c	15.7 c	5.7 d	12.2 c	17.1 b	7.1 c	32.4 c	39.6 cd	25.1 c
Oregon S.	—	—	—	13.0 c	18.4 c	7.6 c	13.7 bc	17.6 b	9.8 b	39.9 b	46.9 bc	32.9 b
Sharpred	—	—	—	7.1 d	10.8 d	3.5 e	7.4 d	10.7 c	4.1 d	22.0 d	26.5 e	17.5 d
Topred	—	—	—	4.9 d	7.6 d	2.3 e	6.4 d	8.5 c	4.3 d	18.8 d	25.5 e	12.1 e
1994				6 August			19 August			31 August		
R. Miracle	12.9 a	19.2 a	6.7 a	26.3 a	33.1 a	19.6 a	36.2 a	48.6 a	23.9 a	47.0 a	61.4 a	32.6 a
E. Red One	5.2 b	9.3 b	1.1 b	13.0 bc	20.4 b	5.6 b	18.5 b	28.1 b	9.0 b	31.5 b	45.9 b	17.1 b
Red Chief	4.2 bc	7.3 b	1.2 b	13.4 bc	20.3 b	6.4 b	19.4 b	30.2 b	8.6 b	31.7 b	48.3 b	15.1 b
Elite	4.0 bc	6.6 bc	1.4 b	8.4 bc	13.1 b	3.7 b	8.8 c	13.7 c	3.8 b	15.9 cd	26.3 d	5.4 cd
Oregon S.	4.8 bc	8.9 b	0.8 bc	11.0 bc	16.5 b	5.4 b	17.9 b	26.4 b	9.4 b	25.4 c	37.5 c	13.4 bc
Sharpred	3.1 cd	5.9 bcd	0.2 cd	10.6 bc	14.9 b	6.1 b	11.8 c	18.8 c	4.8 b	17.1 cd	28.0 d	6.2 cd
Topred	1.4 d	2.7 d	0.01 d	7.2 c	11.3 b	3.1 b	8.3 c	12.7 c	3.8 b	8.9 d	14.5 e	3.4 d

(+) Each value is the mean of BL and BA sides in dates previous to commercial harvest.

(\*) Each value is the mean of BL and BA sides at commercial harvest.

(++) Each value is the mean of one season and one picking date previous to commercial harvest and contains 50 determinations of BS or BA (5 replications of one tree plots and 10 fruits/tree).

(~) Each value is the mean of one season at commercial harvest and contains 75 colour measurements of BS or BA (5 replications of one tree plots and 15 fruits/tree).

y Mean separation within cultivars in the same row for the same year by Duncan's new multiple range test,  $P = 0.05$ , if the F-test was significant in the ANOVA.

Season factor was significant ( $P < 0.01$ ) at commercial harvest.

Season x sampling date interaction was significant ( $P < 0.01$ ).

**Table 3. Growth, cumulative yield, average fruit weight and size, firmness, soluble solids concentrations (SC) and titratable acidity (TA) of seven 'Delicious' strains. Data are the means of 1992, 1993 and 1994 seasons.**

Strain	Growth type <sup>1</sup>	Cumulative yield <sup>2</sup>	Weight <sup>3</sup> (g/fruit)	Fruit size <sup>4</sup> (mm)	Firmness <sup>4</sup> (N)	SSC <sup>5</sup> (%)	TA <sup>6</sup> (g/l)
Red Miracle	St(Sp)	61.4 d	237.2 ab	85.3 a	69.6 ab	12.6 ab	3.9 a
Early Red One	St(Sp)	94.1 a	239.4 ab	78.2 bc	71.4 a	11.8 b	3.3 b
Red Chief	Sp	61.2 d	232.5 ab	80.1 bc	71.5 a	11.6 b	3.3 b
Elite	Sp	55.9 d	244.6 ab	85.6 a	70.5 a	12.2 ab	3.4 b
Oregon Spur	Sp	76.4 c	208.8 b	77.6 c	67.6 b	12.1 ab	3.1 b
Sharpred	St	83.3 b	206.1 b	76.9 c	70.5 a	13.2 a	3.6 ab
Topred	St	58.1 d	257.7 a	84.3 ab	67.6 b	12.4 ab	3.7 a

<sup>1</sup>Growth type: Sp = spur; St = standard.

<sup>2</sup>Cumulative yield over 1992 to 1994 period (kg.tree<sup>-1</sup>). Each value is mean of 5 determinations.

<sup>3</sup>Each value is mean of 75 determinations.

<sup>4</sup>Each value is mean of 150 determinations.

<sup>5</sup>Each value is mean of 5 determinations.

<sup>6</sup>Mean separation within treatments in the same row by Duncan's new multiple range test,  $P = 0.05$ , if the F-test was significant in the ANOVA.

study, Singha et al. (31, 32) and Fisher et al. (15) noted that fruit of high-coloring strains ('Scarlet Spur,' 'Oregon Spur II,' 'Ace,' 'Nured') colored well even in shaded areas of the trees with little light. The anthocyanin distribution on both sides showed that in hot seasons such as 1992 and 1994 color differences between sides were greater than in 1993, because the anthocyanin content in medium or low colored strains was lower, especially on the BA side. On the commercial harvest the ANOVA preformed shows the significance of side factor ( $P \leq 0.05$ ) for anthocyanin content and also for hue angle and L\* (data not shown).

The linear regression of the anthocyanin content on L\*, a\*/b\* and hue angle for all strains at commercial harvest, provided a coefficient of determination ( $R^2$ ) of 0.80 and 0.82 and 0.79 ( $P < 0.009$ ), respectively (Fig.1). The a\*/b\* ratio is directly related to anthocyanin content, whereas hue angle and L\* are inversely related. As reported by Singha et al. (33) with 'Delicious' strains, color changes when fruit darkens to L\* < 50 (Table 1) are closely related to changes in anthocyanin content. These results indicate a good prediction of anthocyanin content based on chromaticity values measured by the colorimeter. As reported by Singha et al. (31) the a\* value (a measure of

redness) *per se* is poorly related to anthocyanin content. Linear regressions showed that the b\* value was significantly higher on the non blushed surface and in the most poorly coloring strains, and this would be expected to influence the a\*/b\* ratio, so this ratio relates far better to the extractable anthocyanin level than do the a\* or b\* values. Given the good relationship between anthocyanin content and chromaticity parameters established above, our study supports the conclusion of Chalmers et al. (8) and Singha et al. (33) who state that it may be possible to use the levels of anthocyanin as an index of maturity in red apple cultivars, excepting highly colored strains such as 'Red Miracle,' because high levels are reached at early stages of fruit development (Table 2).

This study evidences that significant differences exist between strains both on earliness and rate of red color development. Early coloring strains tend to have better developed color at harvest (30). As suggested by Baugher et al. (5) spur-type growth habit was not correlated with high color rating. Monitoring the evolution of parameters such as L\*, Hue angle or a\*/b\* ratio or a combination of these in the period prior to harvest may prove to be a useful harvest guide. Furthermore, the good relationship established between antho-

cyanin content and chromaticity parameters allow the use of portable colorimeter for rapid, non destructive estimation of fruit anthocyanin content in situ.

#### *Yield, fruit weight, fruit size and fruit quality*

Strains were categorized as standard or spur based on their bearing habit according to Warrington et al. (35). 'Early Red One' and 'Red Miracle' because their behaviour similar to spur types were grafted on MM. 106, as the other spur strains. 'Early Red One' had a larger TCSA and 'Elite' and 'Red Chief' the smallest ones (data not shown). The 3-year cumulative yield of 'Early Red One' was the highest, 'Oregon Spur' and 'Sharpred' showed intermediate values and 'Elite', 'Topred', 'Red Chief' and 'Red Miracle' had the lowest (Table 3). There was no particular pattern along the line between standard and spur types for cumulative yield.

Mean values of fruit weight obtained in the three seasons (Table 3) at commercial harvest indicated that 'Topred' was significantly heavier than 'Oregon Spur' and 'Sharpred', but not different from other strains. 'Red Miracle' and 'Elite' were larger than 'Oregon Spur', 'Sharpred', 'Early Red One' and 'Red Chief'; there were no differences between 'Early Red One', 'Red Chief', 'Oregon Spur' and 'Sharpred'. Ketchie (22) also reported no differences in fruit weight and size between 25 'Red Delicious' standard and spur strains ('Early Red One', 'Topred', 'Sharpred' and 'Oregon Spur' included). This finding differs from reports by Baugher et al. (5) and Fallahi et al. (13), in which 'Oregon Spur' was larger than 'Topred', 'Red Chief' and 'Sharpred'. Le Lezec et al. (24) found no differences between 'Topred' and 'Early Red One' nor between these two strains and 'Red Chief', 'Sharpred' and 'Oregon Spur'. This reference conflicts from our results, in which 'Oregon Spur' were smaller and lighter. Although crop load was statistically different (Table 3), the comparison of fruit weight and fruit size between strains was done because crop load alone may not

necessarily alter fruit weight, and the genetic makeup of the strains plays an important role in fruit weight (13).

At commercial harvest 'Oregon Spur' and 'Topred' were significantly softer than 'Early Red One', 'Red Chief', 'Elite', and 'Sharpred', and no different from 'Red Miracle' (Table 3). Similar to our results, other authors (10, 13) reported differences in firmness of various 'Delicious' strains; 'Topred' had lower values than the other strains. Baugher et al. (5) and Singha et al. (32) indicated that it is difficult to establish differences at the harvest date between standard and spur types based on values of firmness, SSC and TA. Among strains 'Sharpred' had higher SSC than 'Early', 'Red One' and 'Red Chief' (spur type), and were no different from 'Elite', 'Red Miracle', 'Oregon Spur' and 'Topred' (Table 3). Fruit of 'Topred' and 'Red Miracle' had higher TA values than 'Early Red One', 'Red Chief', 'Elite' and 'Oregon Spur', but were no, different from 'Sharpred'. High values of both SSC and TA are usually related to better fruit quality. Some of the strains with low yield (data not shown) such as 'Topred' had high SSC at harvest. Some reports related the bearing habit with high SSC content in standard strains, which indicates a higher quality than spur strains (5, 24), although other authors have found no differences in SSC and fruit quality between standard and spur types (13). As suggested by Dozier et al. (12), in our study it was not possible to establish differences associated with the bearing habit (standard or spur type) based on SSC, TA and firmness, although differences between strains were obtained for all quality parameters. These differences depended on strains but also on other factors such as tree age, rootstock, irrigation system and physiological stage of fruit at harvest (9, 20, 5, 13).

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### Paclobutrazol – Cherry

Methods of application to 'Bing' trees were soil drench (SD), row side drench (RSD) and bark painting (BP). SD and RSD showed growth inhibition 12 days before BP trees. Fruit set was reduced by SD and RSD but not by BP and BP had the highest 2 year yield. BP shows promise since it gave growth control and left less soil residue. From Jacyna and Dodds. 1999. *J. Hort. Sci. and Biotech* 74(2):213—13.

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