Effect of rootstock and manual floral bud thinning on organoleptical and nutraceutical properties of sweet cherry (*Prunus avium* L.) cv 'Lapins'

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SUMMARY. – Rootstock selection and thinning treatment have long been shown to positively affect quality parameters of fruits. However, rootstock selection for high content of phytochemical in sweet cherry is in its infancy and no data are available about the effect of manual thinning on these compounds. In Experiment 1, fruit of 'Lapins' grafted onto MaxMa Delbard® 60 Broksec® showed the highest ripening index among the 11 rootstocks evaluated, associated with high anthocyanin, flavonoid content, and total antioxidant ability. In Experiment 2, MaxMa Delbard® 60 Broksec® was selected as rootstock for 25% and 50% of manual bud thinning treatment. Thinning increased significantly fruit size (+9.5% and +19%, respectively), solid soluble and anthocyanin content, and total antioxidant ability of drupes. Chroma and *L** values decreased along with the increment of anthocyanin content. Beside the classic rootstock selection, thinning is a promising approach to further increase both organoleptic and nutraceutical properties of sweet cherry.

INTRODUCTION. – Sweet cherry fruit (*Prunus avium* L.) is one of the widely appreciated spring-summer fruit in temperate areas of Europe, especially in Mediterranean basin. In addition to a large consensus for the excellent organoleptic characteristics, the drupe is also a huge source of phytochemicals (KELEBEK and SELLI, 2011). For that reason, sweet cherry is largely utilized for juice and syrup preparation as well.

Polyphenols are the most abundant phytochemicals of sweet cherry, and certainly anthocyanins are among the most abundant flavonoids in the drupe (USENIK *et al*., 2008; KELEBEK and SELLI, 2011). Flavonoids have long been shown to possess strong antioxidant ability, and some

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studies have demonstrated strong correlations between anthocyanin content and total antioxidant ability of sweet cherry (Serrano *et al*., 2005). Thus, their dietary intake can improve human health and prevent different pathologies such as cancer (Stoner *et al*., 2008), inflammatory (Cicerale *et al*., 2012) and cardiovascular diseases (Gan *et al*., 2010).

In addition to their nutritional role, flavonoids – and in particular anthocyanins – are also responsible for quality characteristics such as flavour, taste, astringency, but are mainly involved in colour development of sweet cherry (Gao and Mazza, 1995). Thus, skin colour and nutraceutical value of sweet cherry are both mainly dependent on the total content of anthocyanins and other flavonoids (Gao and Mazza, 1995). The maximization of these moieties can make the product more suitable to be consumed as whole fruit (Dever *et al*., 1996), but even more to be used as raw material for juice or beverage production. In the first case, a bright red colour can indeed positively attract consumers, while the richness of anthocyanins may confer a bright red colour to the juice, reducing significantly the need of chemical or natural colorants. The latter aspect, coupled with a strong antioxidant activity of anthocyanins, can increase the healthiness of the sweet cherry juice.

The colourimetric CIE system (Commission International d'Eclairage) is widely used in the quantification and characterization of chromatic properties of sweet cherry (Mozetič *et al*., 2004; Gonçalves *et al*., 2007). Despite results obtained by different authors on the chromatic parameters in sweet cherry fruit are sometimes contrasting, different studies have found a negative correlation among anthocyanin content, colour intensity (chroma), while redness and greenness as well as yellowness and blueness seem less related to the anthocyanin content (Mozetič *et al*., 2004; Gonçalves *et al*., 2007).

As for other fruits, the selection of rootstock is a common practice to improve the productivity under specific pedoclimatic conditions (Cantín *et al*., 2010; Usenik *et al*., 2010). Traditionally, sweet cherries are cultivated by grafting on generative rootstocks of *Prunus avium*, *P. mahaleb*, *P. cerasus*, and hybrids of *Prunus* spp. Rootstock evaluation has been very extensive in the last decade, increasing significantly the profitability of fruit production (Remorini *et al*., 2008; Prassinos *et al*., 2009). More recently, breeders have been interested not only in maximizing yield, but also in the selection of rootstocks which give fruits with a high nutraceutical value. However, to the best of our knowledge only few works have explored this topic in sweet cherry (Jakobek *et*

al., 2009; Cantín *et al*., 2010; Usenik *et al*., 2010), and there are no data about rootstock selection for production of sweet cherry fruit whit high levels of phytochemical compounds (i.e. flavonoids, and mainly anthocyanins among them).

In addition to the rootstock selection, the thinning treatment is another widely applied agronomical techniques in fruit growing to improve fruit yield and quality. Usually the technique has been addressed to maximize the fruit size, despite other features (firmness, flavour, storability) could be achieved by flower or flower bud thinning. The thinning treatment can be performed either by chemical treatments such as ammonium thiosulfate (Whiting *et al*., 2006) (ATS) and gibberellic acid (Lenahan *et al*., 2006) (GA) or mechanically. Mechanical thinning is an environmentally friendly technology and an alternative to the standard chemical thinning, which is limited by the decreasing number of registered chemical compounds which success is very dependent on weather conditions, variety, flowering dynamics and tree age (Solomakhin and Blanke, 1996). In addition, with the use of GA – one of the most utilized thinning chemical agents – the colour development of fruit can be delayed as gibberellic acid has been recognized as responsible for the repression of the anthocyanin biosynthesis during fruit development (Cline and Trought, 2007). Conversely, the mechanical thinning has exerted positive results in terms of anthocyanin accumulation in apple fruit (Solomakhin and Blanke, 1996) and it could overcome the problem of the retardation of skin pigmentation. Noteworthy, in other species some devices have already been engineered for mechanical flower thinning (Solomakhin and Blanke, 1996 and references therein) and their use could be also extended to sweet cherry, making the treatment less time consuming and more economically sustainable than the manual thinning.

Whilst in other fruits, such as apple and peach, the thinning effects are well documented, in sweet cherry research on flower thinning is in its infancy. Indeed, there are some works which have demonstrated that chemical flower thinning of sweet cherry can increase the drupe size and weight (Whiting *et al*., 2005; Lenahan *et al*., 2006) as well as the yield of large fruits (Whiting *et al*., 2006), but the results are not consistent with those of other authors. For example, Schoed *et al.* (2009) did not find any significant difference in fruit size and weight in four different cultivars of sweet cherry, while the influence of thinning on the content of solid soluble was genotype-dependent. In addition, based to the best of our knowledge, there is a lack of information concerning

the influence of thinning on the anthocyanin content and the total antioxidant activity of fruits.

The goal of this work is double and separated in two independent experimental trials: in Experiment 1 we screened sweet cherry cv 'Lapins' grafted on 11 rootstocks to found the most suitable one(s) which maintained a good trade-off between solid soluble content (SSC) and titratable acidity (TA) associated with high anthocyanin and flavonoid contents, and free radical scavenging ability. In Experiment 2 we tested the hypothesis that manual floral bud thinning prior to blooming positively impacts the fruit features selected in Experiment 1. We consider the selection of rootstocks addressed to improve the nutraceutical values of sweet cherry as a novel criterion of selection, different from the classic approach aimed to maximize yield and/or fruit size. In addition, we are not aware of any study about thinning carried on in sweet cherry addressed to evaluate the effect on the anthocyanin concentration and antioxidant capacity, and thus we consider Experiment 2 as the 'core of novelty' of this experimental work.

MATERIALS AND METHODS. – *Plant material and experimental design*. – Experiment 1 and 2 were performed during Spring 2008 and 2009, respectively, at the experimental orchard of the Department of Agriculture, Food and Environment of the University of Pisa located in Colignola (Pisa, Italy, 43°43' N 10°23' E). The sweet cherry orchard was planted in February 2001 and trained in a spindle system with a distance of 5 × 5 m between trees.

For Experiment 1 a total of 33 trees, grafted on 11 rootstocks of different vigour and genetic origin (Table 1) were randomly selected. Conventional commercial irrigation, fertilization and pruning treatments were carried out. At the standard commercial stage (June 5th) for 'Lapins', fifty fruits were harvested from each tree and fruits were analysed for organoleptic features, flavonoid and anthocyanin contents and antioxidant activity.

For Experiment 2, a total of nine trees grafted on MaxMa Delbard®60 Broksec* rootstock were randomly selected. Floral buds were not thinned in 3 trees, while in another 3 trees 50% of the total buds were manually thinned, and in the last three ones 25% were hand-thinned (April 4th). Harvesting time was similar to that of Experiment 1 (first decade of June).

Organoleptic features assessment. – Fresh weight (fw; expressed as g), SSC (°Brix), and TA (% malic acid) were determined immediately

TABLE 1. – *Genetic origin and classification of rootstocks.*

Rootstock	Genetic origin	Classification
CAB 6P	*P. cerasus*	semi-vigorous
CAB 11E	*P. cerasus*	semi-vigorous
Colt	*P. avium* x *P. pseudocerasus*	vigorous
Gisela® 6*	*P. cerasus* x *P. canescens*	dwarfing
MaxMa Delbard® 14 Brokforest	*P. mahaleb* x *P. avium*	semi-vigorous
MaxMa Delbard® 60 Broksec*	*P. avium* x *P. mahaleb*	vigorous
Mazzard F12/1	*P. avium*	vigorous
Pontaleb® - Ferci* SL 405	*P. mahaleb*	vigorous
SL 64	*P. mahaleb*	vigorous
Tabel® Edabriz	*P. cerasus*	dwarfing
Weiroot 53®	*P. cerasus*	dwarfing

after harvest. Fruit juice SSC from each sample was measured using a digital refractometer (Model 53011, T.R., Forlì, Italy). To determine TA, fruit juice samples were diluted with deionized water (1:10) and microtritated to pH 8.1 with 0.1 N NaOH and expressed as malic acid percentage. The ripening index (RI) was calculated as SSC/TA ratio.

Colour analyses. – Skin colour measurements were carried out in 15 fruits using standard CIELab colour space coordinates determined using an Ocean Optic HR2000-UV-VIS-NIR spectrometer coupled whit a tungsten halogen DH2000 light source (Ocean Optics, USA). L* represents the lightness of colours, a^* value represents redness and greenness (a^* and $-a^*$, respectively) and b^* value represents yellowness and blueness (b^* and $-b^*$, respectively). These values were used to calculate chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$), which indicates the intensity or colour saturation.

Spectrophotometric determination of total flavonoid and anthocyanin contents. – Spectrophotometric analyses of total anthocyanin and flavonoid contents were performed as follow: 2 g of stoned sweet cherry fruit were grounded with 10 mL of 80% aqueous acidified ethanol (5% HCl v/v). Sweet cherry juice was filtered through a Whatman No. 41 filter paper (Sigma-Aldrich, Milan, Italy), collected and keep under the dark at 4°C overnight. The pulp which remained suspended over the filter paper was washed with further 90 mL of 80% aqueous acidified ethanol and shaken overnight at 4°C. Sweet cherry juice (10 mL) and

the pulp washing solution (90 mL) were collected together in a single sample and the absorbance was spectrophotometrically assessed at 535 nm for total anthocyanins and at 425 nm for total flavonoids using an Ultrospec 2100 Pro spectrophotometer (GE Healthcare Ltd, Little Chalfont, England). For total flavonoid content, 2% $AlCl_3$ (w/v) in methanol solution was added to the sample prior the spectrophotometric determination. Absorbance values for anthocyanins were converted to cyanidin-3-glucoside equivalents (CGE) kg^{-1} fw and quercetin equivalents (QE) kg^{-1} fw. The calibration curves were obtained by measuring absorbance of standard solutions of pure cyanidin-3-glucoside (Extrasynthese, Genay, France) and quercetin (Sigma-Aldrich, Milan, Italy) dissolved in the same solvent.

DPPH scavenging ability. – The antioxidant activity of each sample was determined using a modified version of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging assay as described by Kim *et al.* (2005). Methanolic sweet cherry extract (20 µL) was diluted to 100 µL with 80% aqueous methanol and added to 0.4 mL of 0.1 M Tris-HCl buffer and 0.5 mL of 0.3 mM DPPH in methanol. The solution was thoroughly mixed and incubated in the dark for 20 min at room temperature. The absorbance of the sample mixture (A_{sample}) was monitored at 517 nm *versus* a methanol/Tris-HCl blank. The absorbance of a control sample ($A_{control}$) containing only methanol/Tris-HCl and DPPH was also analysed. The % DPPH free radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH free radical scavenging} = [1 - (ABS_{sample}/ABS_{control})] \times 100$$

The antioxidant activity was determined by comparing the % DPPH free radical scavenging of each sample to a calibration curve prepared with Trolox, a well-known antioxidant standard. Antioxidant activity was expressed as Trolox equivalents (TE; mmol TE kg^{-1} fw) to allow direct comparison of the free radical scavenging capabilities among all samples.

Statistical analysis. – Reported data are the means (±SD) of at least five independent replicates (n = 5). For determination of fruit weight and skin colour means are representative of 15 replicates (n = 15) for each sample. Means were compared by one-way ANOVA following Bartlett's test to assess the homogeneity of variance among samples. Percent values were arcsin transformed prior to analyses. Differences among means were evaluated by Fisher's least-significant difference test

(LSD) for P = 0.05. Linear correlations were calculated between total anthocyanins *versus* total antioxidant ability (DPPH test), and flavonoid content *versus* total antioxidant ability. Only significant correlation with $R^2 < 0.90$ were reported. All statistical analyses were performed using CoStat software package (CoHort™ Software, Berkeley, CA, USA).

RESULTS AND DISCUSSION. – *Experiment 1.* – The effect of grafting resulted in a high variability of fruit weight, SSC, TA and RI (Table 2). The weight of a single drupe ranged from 4.1 g using Tabel® Edabriz as rootstock to 8.1 g when sweet cherry cv 'Lapins' was grafted onto CAB 6P. Mean value approximated 6.6 g among all the rootstocks. 'Lapins' grafted on MaxMa Delbard® 14 and MaxMa Delbard® 60 resulted in fruits with a weight closer to the mean value (6.8 and 6.7 g fruit^{-1}, respectively). A high variability in fruit weight of sweet cherry 'Lapins' was also found by GRATACOS *et al.* (2007) using six rootstocks among those tested in this experiment. In agreement with the results obtained in this experiment, the latter authors found that CAB 6P and Colt were the rootstocks which yielded weightier fruits, while Gisela 6 yielded the smallest ones. Thus, vigour of the rootstock seems sometimes positively

TABLE 2. – *Organoleptic parameters of sweet cherry fruits cv 'Lapins' grafted onto different rootstocks.*

Rootstock	Fruit weight	SSC	TA	Ripening index
CAB 6P	8.1 (0.7) a	14.2 (0.7) cd	0.52 (0.05) a	27.7 (0.9) e
CAB 11E	7.5 (0.6) ab	13.1 (0.8) ef	0.48 (0.03) b	27.9. (0.8) e
Colt	7.3 (1.0) ab	14.0 (1.3) cd	0.39 (0.01) de	34.7 (1.4) bc
Gisela® 6*	4.9 (0.8) ef	14.5 (0.9) bc	0.40 (0.06) d	35.9 (1.6) b
MaxMa Delbard® 14	6.8 (0.8) bc	12.5 (0.7) f	0.53 (0.05) a	24.2 (0.8) f
MaxMa Delbard® 60	6.7 (0.6) bc	14.1 (0.8) bc	0.35 (0.03) ef	40.6 (0.7) a
Mazzard F12/1	7.1 (0.9) bc	14.4 (0.8) bc	0.49 (0.07) b	30.3 (1.7) d
Pontaleb® - Ferci* SL 405	6.3 (0.8) cd	15.0 (1.0) ab	0.38 (0.04) f	39.5 (1.8) a
SL 64	7.0 (0.6) bc	15.4 (0.9) a	0.44 (0.05) c	34.8 (1.2) b
Tabel® Edabriz	4.1 (1.0) f	13.5 (0.8) de	0.41 (0.06) d	33.4 (1.0) c
Weiroot 53®	5.8 (0.7) de	14.4 (1.0) bc	0.48 (0.09) b	30.3 (1.7) d

Means (±SD) are representative of 15 replicates for fruit weight and 5 replicates for SSC, TA, and RI. Fruit weight is expressed in grams. SSC, soluble solid content (°Brix); TA, titratable acidity (% malic acid); RI, ripening index (SSC/TA). Means were compared by one-way ANOVA with rootstock as source of variability. Means followed by the same letter are not significantly different after Fisher's least-significant difference test (LSD).

influence the fruit size. In other cases fruit size is also dependent on pedoclimatic attributes, and in other experimental conditions also dwarfing rootstocks yielded fruits with reasonably comparable weight of that yielded using (semi)vigorous rootstocks (USENIK *et al*., 2010).

Sweet cherry grafted on SL 64 showed the highest SSC (15.4 ± 0.9 °Brix), while the lowest values were found using MaxMa Delbard® 14 (12.5 ± 0.7 °Brix). Values of TA ranged from 0.35 (MaxMa Delbard® 60) to 0.52 and 0.53% malic acid (CAB 6P and MaxMa Delbard® 14, respectively). Noteworthy, the highest value of RI, a key parameter for assessing sweet cherry quality, was found in MaxMa Delbard® 60 (40.6 ± 0.7). Values of SSC, TA and RI found in this work are comparable to those found in fully-ripe fruits by other authors (SCHOEDL *et al*., 2009; USENIK *et al*., 2010). The high variability found within the fruit organoleptic parameters previously mentioned could be considered an inherent prerogative of the genetic differences among the different rootstocks, as confirmed by other researches on sweet cherry cv 'Lapins' (JAKOBEK *et al*., 2009; USENIK *et al*., 2010). To further complicate the picture, the seasonal variations may significantly affect sweet cherry organoleptic parameters as well (WHITING and OPHARD, 2005; WHITING *et al*., 2006; SCHOEDL *et al*., 2009; CANTÍN *et al*., 2010). In addition, in agreement with our results, the influence of rootstock can also results in a different accumulation of phytochemicals in fruits as reported below.

The concentration of anthocyanins in 'Lapins' sweet cherry fruits ranged from 0.1 (MaxMa Delbard® 14) to 0.85 g CGE kg^{-1} fw (Weiroot 53®). Thus, anthocyanin content resulted largely affected by the rootstock selected for grafting (Fig. 1A) as confirmed by researches either in 'Lapins' (JAKOBEK *et al*., 2009; USENIK *et al*., 2010) or in other sweet cherry cultivars (CHAOVANALIKIT *et al*., 2004; GONÇALVES *et al*., 2007; LIU *et al*., 2011). Conversely, accumulation of total flavonoids and total antioxidant ability were far less heterogeneous on the basis of the rootstock utilized, despite many differences occurred as well (Figs. 1B and C). For all the nutraceutical properties investigated (anthocyanin, flavonoid content and antioxidant ability), fruits grafted on MaxMa Delbard® 60 never showed the highest values.

Given that flavonoids, and anthocyanins among them, have long been demonstrated to possess strong antioxidant ability (IGNAT *et al*., 2011; WALLACE, 2011), the selection of rootstock(s) which allow fruits to yield high content of phytochemicals could proceed in parallel with the selection for fruit size and tree yield. In Experiment 1, we found

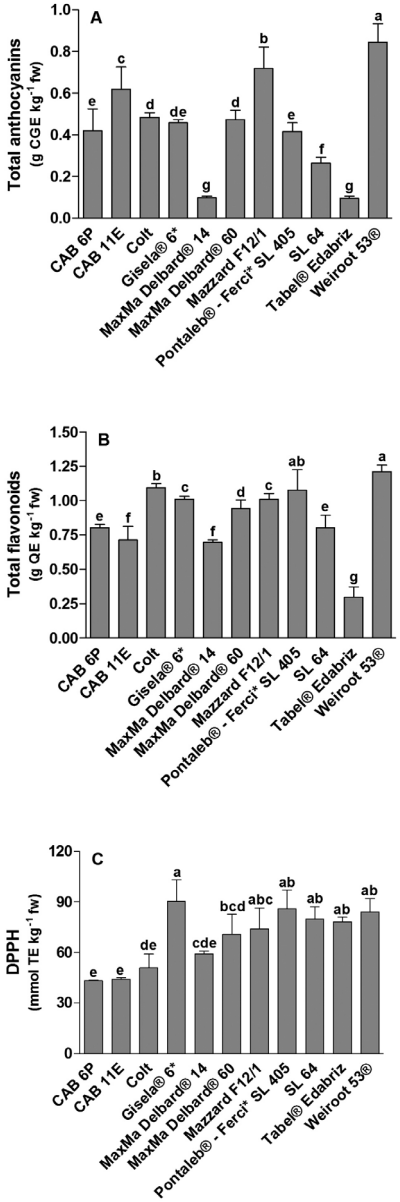

Fig. 1. – Total anthocyanin (A), flavonoid content (B), and total antioxidant ability (C) in sweet cherry cv 'Lapins' grafted on 11 different rootstocks. Means ($n = 5$; ±SD) followed by different letters are significantly different at P = 0.05 according to LSD test following one-way ANOVA. CGE, cyanidin-3-glucoside equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; QE, quercetin equivalents; TE, Trolox equivalents.

significant correlations between total anthocyanin and flavonoid contents *versus* total antioxidant ability (DPPH) for most of the rootstocks utilized. However, in Table 3 we reported only highly significant correlations with $R^2 < 0.90$ in order to simplify the criteria of selection for the rootstock most suitable for Experiment 2. Rootstock CAB 11E and MaxMa Delbard® 60 were the only two whose fruits had a high and significantly positive correlation both for anthocyanins ($R^2 = 0.97$ and 0.94, respectively; $P < 0.05$) and flavonoids ($R^2 = 0.98$ for both; $P < 0.05$). A strong correlation between anthocyanins and total antioxidant ability was also previously observed by Liu *et al*. (2011) in 12 different sweet cherry genotypes, 'Lapins' being among them. Conversely, in disagreement to our data, the same research did not show any significant correlation between total flavonoids and antioxidant ability of fruits, whilst other authors found similar results as those reported in this study (Prvulovic *et al*., 2011).

At the end of Experiment 1, we selected MaxMa Delbard® 60 as the most suitable rootstock for the flower bud thinning experiment (Experiment 2) due to: (i) the highest RI found among all the rootstocks (with Pontaleb® - Ferci® SL 405); (ii) the highly significant positive correlation among anthocyanin, flavonoid content and DPPH values; (iii) the higher values of flavonoids and total antioxidant ability compared to

Table 3. – *Correlation among anthocyanins, flavonoids and total antioxidant ability of fruits.*

Rootstock	Anthocyanins	Flavonoids
CAB 6P		
CAB 11E	0.97*	0.98*
Colt		
Gisela® 6®	0.98**	
MaxMa Delbard® 14		
MaxMa Delbard® 60	0.94*	0.98*
Mazzard F12/1		
Pontaleb® - Ferci® SL 405		
SL 64	0.99**	
Tabel® Edabriz		
Weiroot 53®	0.93*	

Values represent the R^2 value of each significant linear correlation. * $P < 0.05$; ** $P < 0.01$, $P < 0.001$.

CAB 11E (the other rootstocks with a high correlation between anthocyanin *versus* DPPH, and flavonoids *versus* DPPH). Briefly, MaxMa Delbard® 60 insured the best compromise between organoleptic and nutraceutical properties of fruits. Noteworthy, MaxMa Delbard® 60 was classified among the tolerant rootstocks for pollenborne viruses prune dwarf (PDV) or prunus necrotic ringspot (PNRSV) (Lang, 2000).

Experiment 2. – As expected, the first evident effect of thinning was the increment of fruit weight (about +9.5% by 25% thinning and +19% by 50% thinning; Table 4). The increment of fruit weight and size is one of the most desired effects of thinning treatment and it has been previously reported by other researches in sweet cherry (Whiting and Ophard, 2005; Lenahan *et al*., 2006; Whiting *et al*., 2006). However, the latter results were achieved using chemical flower thinners while, to the best of our knowledge, the effect of mechanical/manual bud thinning has never been reported in sweet cherry. In addition, not all the chemical thinners have demonstrated positive effect on fruit size increment (Whiting *et al*., 2006; Schoedl *et al*., 2009). Mechanical treatment was also positively correlated with higher fruit size in other species such as apple (Solomakhin *et al*., 2010; Henriod *et al*., 2011). The positive effect of thinning is basically attributable to the balance of fruit/foliage ratio, whereas excessive yield may lead to small fruit size (Webster and Spenced, 2000).

In this experiment, manual thinning induced a significant increment of SSC as well (13.8 °Brix in not-thinned plants *versus* 15.4 and 15.7 °Brix with 25% and 50% thinning, respectively; Table 4). Values of TA remained statistically unchanged as well as the RI. Scoedl *et al.* (2009) found no improvement of SSC and TA with ATS-based flower thinning in 4 sweet cherry cultivars, despite in a few cases enhancement of SSC was observed (e.g., in cv 'Techlovan' and 'Merchant' using 17.6 and 1.9 g ATS L^{-1}, respectively). Conversely, the increment of SSC found by Lenahan and Whiting (2006) with applications of 50 and 100 mg L^{-1} GA (+7% and 12% higher SSC, respectively) is in agreement with our findings.

Thinning treatment also positively affected the total anthocyanin content (+14.0% and +19.5% with 25% and 50% thinning, respectively; Fig. 2A). The decline of b^*, chroma, and L^* found in fruits harvested from thinned plants (Table 4) appeared well related to the increased anthocyanin content as reported by other authors (Mozetič *et al*., 2004; Gonçalves *et al*., 2007). Conversely, a^* was not significantly affected by thinning in accordance with Mozetic *et al*. (2004) who underlined

TABLE 4. – *Organoleptic and colorimetric parameters of sweet cherry fruits cv 'Lapins' grafted on MaxMa Delbard® 60 rootstock after bud thinning.*

Parameter	No thinning	25% thinning	50% thinning
Fruit weight	6.7 (0.2) c	7.4 (0.5) b	8.2 (0.6) a
SSC	13.8 (0.4) a	15.4 (0.6) b	15.7 (0.3) b
TA	0.38 (0.05) a	0.41 (0.10) a	0.43 (0.03) a
Ripening index	38.3 (0.4) a	38.9 (0.7) a	37.8 (0.8) a
*L**	22.4 (7.3) a	17.6 (4.8) b	17.7 (3.6) b
*a**	40.1 (5.8) a	39.6 (5.4) a	37.0 (4.4) a
*b**	23.7 (11.8) a	19.3 (11.2) b	11.5 (4.4) c
Chroma	47.4 (9.9) a	44.9 (9.0) ab	38.8 (5.3) b

Each value represents the mean (±SD) of 15 replicates for fruit weight and chromatic parameters, and 5 replicates for SSC, TA, and RI. Fruit weight is expressed in grams. SSC, soluble solid content (°Brix); TA, titratable acidity (% malic acid); RI, ripening index (SSC/TA); *L**, *a**, *b**, chroma, colorimetric parameters of skin. Means were compared by one-way ANOVA with thinning as variability factor. Means followed by the same letter are not significantly different after Fisher's least-significant difference test (LSD).

as chroma and *L** appeared to be optimal indicators of anthocyanin accumulation during maturation. It seems logic that yellowness (*b**) and lightness (*L**), and consequently chroma values, may negatively correlate with the anthocyanins levels (GONÇALVES *et al*., 2007), but it is more complex to understand why an increase in pigments causing redness gives lower redness value readings, i.e. decreased *a** values. The phenomenon can be explainable as follow: accumulation of anthocyanins in early stages positively correlate with *a** value (GONÇALVES *et al*., 2007), but in the last stages of drupe ripening the development of dark-red to almost blackish pigments creates an "inversion area", where the increment of anthocyanins concentration (in particular cyanidin-3-glucoside) failed to correlate with *a** (MOZETIČ *et al*., 2004). In brief, this phenomenon is presumed to occur when increased pigment concentration both darkens the fruit and reduces *L**. Thus, we confirmed that instrumentally evaluated reflectance skin colour can be considered suitable for monitoring anthocyanin accumulation, and consequently as a non-destructive method to assess the ripening stage of fruits (DRAKE *et al*., 1982). On the other hand, flavonoid content was significantly unchanged, irrespectively of treatments (Fig. 2B). The fact that pattern of total flavonoids did not follow that of anthocyanins suggests that in addition to anthocyanins other flavonoids in the total pool, such instance flavonols, were differently regulated by thinning.

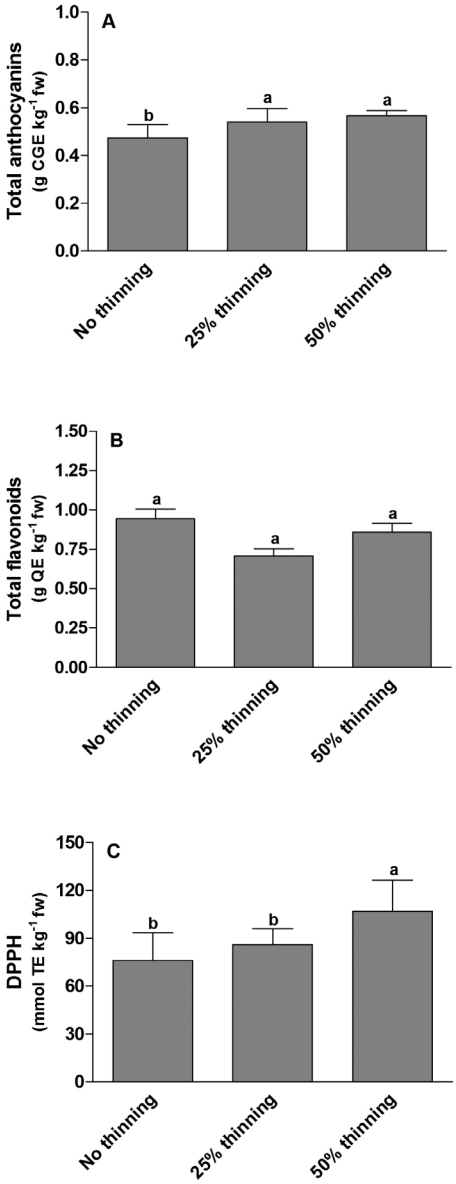

Fig. 2. – Total anthocyanin (A), flavonoid content (B), and total antioxidant ability (C) in sweet cherry cv 'Lapins' grafted onto MaxMa Delbard® 60 rootstock in no thinned plants or after 25% and 50% floral bud thinning. Means (n = 5; ±SD) followed by with different letters are significantly different at P = 0.05 according to LSD test following one-way ANOVA. CGE, cyanidin-3-glucoside equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; QE, quercetin equivalents; TE, Trolox equivalents.

The 50% bud thinning translated to an increased total antioxidant ability of fruits, while the DPPH values for the 25% thinning treatment were only slightly (and not significantly) higher than controls (Fig. 2C). This confirms the powerful antioxidant activity of anthocyanins in sweet cherry fruits, albeit it is undeniable that the total antioxidant capacity of fruit is also dependent on other phytochemicals such as flavonoids or even phenolic acids (Serrano *et al*., 2005).

Our results are also in agreement with those reported for apple fruits after thinning (Solomakhin and Blanke, 2010). Indeed, in that study 'Gala Mondial' apples showed a rise of 17% of total anthocyanin content after thinning, and the authors hypothesized it was attributable to an improved assimilate partitioning within the tree as well as to a more incident sunlight on exposed fruit peel. It is indeed well know that anthocyanin metabolism is strictly influenced both by sugar availability and light exposure (Lancaster and Dougall, 1992). Thus, the higher SSC we found in this work in fruit belonging to thinned branches could have contributed to anthocyanin accumulation in sweet cherry fruit. However, Awad *et al.* (2001) demonstrated that assimilate availability is not always a major regulatory factor in flavonoids and anthocyanins accumulation in 'Red Elstar' apple fruits given that the concentration of those compounds was not affected by crop load. This is a further prove of the complexity of flavonoid accumulations in fruits and it can explain the reason on the basis of the unchanged values of total flavonoids found in Experiment 2. Based on results of this study, we consider the manual thinning of sweet cherry a suitable strategy to positively influence both the organoleptic and nutraceutical properties of fruit.

Conclusions. – We consider the selection of rootstocks that provide high content of phytochemicals in sweet cherry, in particular high levels of anthocyanins, a promising approach to further increase the capacity to attract consumers, and thus the profitability of this fruit. This type of selection can proceed in parallel and without any interference with the classic selection of rootstocks for fruit size or tree yield. Manual (or mechanical) bud thinning can be a useful agronomic technique to further increase the size of fruits and to yield fruits with higher nutraceutical properties *in sensu lato*. Noteworthy, reflectance skin colour assessment is definitely a useful tool to monitor sweet cherry fruit ripening and, in parallel, fruit quality with the privilege of being a non-destructive method. To conclude, we consider this work a pioneering study even

though further research is necessary to confirm this preliminary results, for example using other rootstocks and/or cvs of sweet cherry.

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