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Cultivar effect on the sweet cherry antioxidant and some chemical attributes

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ABSTRACT

This research was carried out to evaluate the phenolic composition, antioxidant capacities, sugars and organic acids content of sweet cherry cultivars (Bianca, Burlat, Johanna 1, Johanna 2, Kordia, Kunzes Kirsche, Merton Premier, Napoleon, Orleans, Regina, Rivan, Schneiders Spate Knorpelkirsche, Summit, Trebnitzer Lotkirsche) grown in Poland. Significant differences were observed between tested cultivars for all studied parameters.

The sum of total soluble solids ranged from 14.3 ('Burlat') to 20.9 g 100 g^{-1} FW ('Bianca') and that of organic acids from 0.43 ('Burlat') to 0.76 g 100 g^{-1} FW ('Napoleon'). Fruit of Bianca cultivar showed the highest levels of anthocyanin (108.5 mg 100 g^{-1} FW) while 'Napoleon' and 'Kunzes Kirsche' contained the lowest levels (1.5 and 1.8 mg 100 g^{-1} FW, respectively). Total phenolic contents ranged from 101 ('Napoleon') to 558 ('Bianca') mg 100 g^{-1} FW, tartaric esters from 26.2 ('Summit') to 66.5 ('Bianca') mg 100 g^{-1} FW and flavonoids from 7.9 ('Summit') to 49.1 ('Bianca') mg 100 g^{-1} FW. Bianca cultivar has also the highest free radical scavenging activity assayed by ABTS and DPPH methods 88 and 90% respectively.

Key words: anthocyanins, organic acids, phenolic compounds, *Prunus avium* L., quality, sugars

INTRODUCTION

The sweet cherry (*Prunus avium* L.) belong to the group of the earliest fruit crops in the temperate regions. They are also one of the most appreciated fruit in the world due to unique taste and other sensory properties. Consumers' acceptance is raised by the knowledge of high content of many phytonutrients and bioactive compounds which may significantly contribute to a healthy diet (Crisostoso et al. 2003, Kim et al. 2005, Fazzari et al. 2008).

Describing the value and quality of sweet cherry fruit phenolic compounds, sugars and organic acids are often mentioned – composition, concentrations and ratios. Some studies published during last years indicate phenolic ability to act as antioxidant agents. Epidemiological and *in vitro* studies show phenolic involvement in reducing risk of cancer, arthritis and other diseases or protective effects on neuronal cells. Consumption of sweet cherries has been specifically associated with the reduction of proliferation of some forms of cancer (Kim et al. 2005, Serrano et al. 2005, Fazzari et al. 2008, Usenik et al. 2008, Kelebek and Selli 2011, McCune et al. 2011).

Anthocyanins are the major group among phenolics in sweet cherries and are well known as responsible for fruit colour. The dominant anthocyanin in sweet cherries is 3-*O*-rutinoside

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of cyanidin, and it accounted for the largest share of the total anthocyanin contents (from 90% to 92%). It is postulated that anthocyanins apart from other phenolics in sweet cherries possess unique ability to scavenge oxygen free radicals and other reactive species. Special interest has been shown for anthocyanins of those fruits since the end of 20th century because of antioxidant properties (Gao and Mazza 1995, Mozetič et al. 2002, Gonçalves et al. 2007, Serrano et al. 2005, Usenik et al, 2008, Serra et al. 2011). Total phenolics could be divided into tartaric esters, flavoniods and antocyanins (Fukumoto and Mazza 2000). Other studies identified as separate groups: phenolic acids, flavonols and flavan-3-ols. At given class specific compounds were already extensively described for sweet cherries (Gonçalves et al. 2004, Mozetič et al. 2006, Usenik et al. 2008). Veberič and Štampar (2005) assayed the content of chlorogenic acid, rutin and epicatechin and found that great differences existed due to the cultivar effect.

Sugars are the major components of sweet cherries, affecting soluble solids and sweetness of fruits. Glucose, followed by fructose, sorbitol and sucrose, are present in the largest amounts in sweet cherries. Sorbitol share is considerable 15-20% of total sugars. It is claimed that sorbitol, one of the sugar alcohols, is more beneficial than other sugars with regard to diet control and dental health (reducing caloric intake) and that it improves the sweet taste and texture of fruits. The sweet cherry organic acid content and composition are of interest because of influence on the sensory properties. The main organic acid of sweet cherries is malic, followed by citric acid (Girard and Kopp 1998, Usenik et al. 2008, Kelebek and Selli 2011, McCune et al. 2011, Hayaloglu and Demir 2015).

Sweet cherry is becoming a commercially important fruit crop in Poland. Annual production was 48.1 thousand tones in 2014. During last four years a 15% increase in production is observed and it is expected to continue in next seasons. Almost 50% of sweet cherry produced in Poland is exported to Germany and Russia. The world leaders in sweet cherry production are Turkey 438 T tones followed by USA 303 T tones in 2011. Almost in all counties in the world an increase in cherry production is observed. The highest rate of increase during last 5 years was noted in Chile 800%. The world's sweet cherry production reached 2 294 T tones in 2013 from 2 072 in 2010 (FAOSTAT 2016).

Several studies have been reported on the physical, chemical, pomological and nutritional properties of sweet cherry in some countries (Girard and Kopp 1998, Vursavuş et al. 2006, Radicevic et al. 2008, Kalyoncu et al. 2009). There has been no detailed research on the chemical attributes of sweet cherries grown in Poland. In this paper we focus on the quantification of organic acids, sugars, phenolic compounds and antioxidant capacity of several cultivars. Only few of evaluated cultivars were studied in other countries and in above mentioned studies.

MATERIAL AND METHODS

Experimental design

Fourteen sweet cherry cultivars (Bianca, Burlat, Johanna 1, Johanna 2, Kordia, Kunzes Kirsche, Merton Premier, Napoleon, Orleans, Regina, Rivan, Schneiders Spate Knorpelkirsche, Summit, Trebnitzer Lotkirsche) grown in Krakow region (south of Poland) were used in this study. 12-years-old sweet cherry trees at Krakow Agricultural University's experimental orchard located in Garlica village (latitude: 52°25′N, longitude: 32°52′E and 280 m over see level) were used. Trees were planted at a spacing of 4 m × 5 m and trained to the central leader system. All maintenance practices (fertilization, plant protection etc.) within experimental orchard was realized according to standard commercial recommendations. The experiment was carried out in the 2011 year. The meteorological data in the experimental year are presented in Figure 1.

Samples were taken from four different branches from three trees of each cultivar. Harvest involved a random sampling from five trees for each cultivar. Samples encompassed 5 kg of cherry from each cultivar. They were kept in cooled bags during transportation to the laboratory.

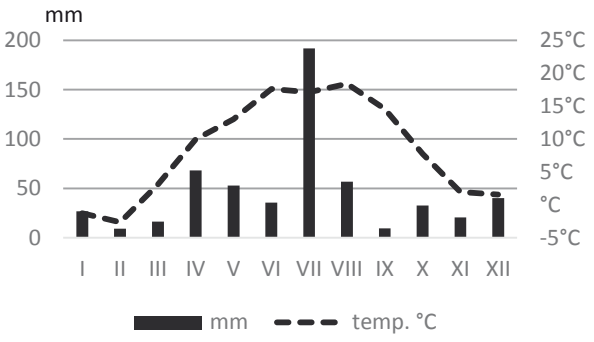

Figure 1. Temperatures and precipitation in year 2011

Chemicals

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulphate and 2,2-diphenyl-1-picryl hydrazyl (DPPH•) was obtained from Sigma-Aldrich (St Louis, MO, USA) (Re et al. 1999). Chlorogenic acid, caffeic acid, quercetin, and cyanidin 3-rutinoside were purchased from Sigma-Aldrich (Steinheim, Germany).

Extraction and determination of sugars, organic acids and pH

Samples were prepared according to general laboratory procedures. Fruits were washed, dried, pitted and homogenized with a manual blender. Homogenate was used as first step for several analyses as listed below.

Mashed (10 g) fruit was dissolved with 100 cm^3 of distilled water. Active acidity expressed as pH of each sample was measured using a pH meter (Jenway, UK). Total acidity of sweet cherry samples were determined by titration method using 0.1 M NaOH. Manual press was used to obtain clear juice from homogenized fruit for sugar evaluation. Sugar content was measured as total soluble solids (TSS) using digital refractometer (Atago PR 100, Japan).

Extraction and analysis of phenolic compounds

Samples were prepared according: 2.5 grams of mashed sample was extracted for 30 minutes with 10 cm^3 methanol containing 1% HCl in dark. Next sample was centrifuged at 12 000 **g** for 7 min at 10°C. The supernatant was filtered through a 0.45 μm cellulose filter, transferred into a glass vials. The quantities of the different phenolic compounds were assessed by method described by Fukumoto and Mazza (2000). Briefly, the method consisted of mixing 0.25 cm^3 of sample with 0.25 cm^3 of 0.1% HCl in 95% ethanol and 4.55 cm^3 of 2% HCl. The absorbance of the solution was then read at 280, 320, 360, and 520 nm to measure total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. Standards used were chlorogenic acid, caffeic acid, quercetin, and cyanidin 3-rutinoside for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. Standards were prepared in 80% methanol except for quercetin, which was prepared in 100% methanol. Abbreviated forms will be used in further paragraphs: CHAE for chlorogenic acid equivalents, CAE for caffeic acid eqivalents, QE for quercetine equivalents and CRE for cyaniding rutinoside equivalents. Concentrations were expressed as milligrams per 100 g FW (Gonçalves et al. 2004, Kelebek and Selli 2011). Triplicate analyses were performed for each sample.

Evaluation of antioxidant activity by the ABTS and DPPH methods

Phenolic extracts were obtained from fruits as mentioned in previous paragraph. Antioxidant activity was measured using ABTS method as described by Re et al. (1999). The ABTS radical cation solution was prepared through the reaction of 7 mm ABTS and 2.45 mm potassium persulphate, after incubation at 23°C in the dark for 12-16 h. The ABTS solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. ABTS solution (3.9 cm^3; absorbance of 0.700 ± 0.005) was added to 0.1 cm^{-3} of the test sample and mixed vigorously. The reaction mixture was allowed to stand at 23°C for 5 and 30 min and the absorbance at 734 nm was immediately recorded. Results are expressed in % of absorbance change against blank sample. Triplicate analyses were run for each sample. Antioxidant activity was determined in further step using the DPPH method as reported by Brand-Williams et al. (1995). An aliquot of 0.1 cm^3 of diluted sample was added to 3.9 cm^3 of DPPH• solution in methanol (6×10^{-5} m). A control sample, containing the same volume of solvent in place of extract, was used to measure the maximum DPPH absorbance. After the reaction was allowed to take place in the dark for 5 and 30 min, the absorbance at 515 nm was recorded to determine the concentration of remaining DPPH. Results are expressed in % of absorbance change against blank sample (Macheix et. al. 1990, Sanchez-Moreno 1998, Pellegrini et. al 2003).

Statistical analysis

The results were compared by the analysis of variance (ANOVA) and Pearson correlation coefficients using Statistica. Duncan's multiple-range tests were used to compare the significant differences of the mean values at $p < 0.05$.

RESULTS AND DISCUSSION

pH values of studied sweet cherries are listed in Table 1. Significant differences were found between the studied cultivars. Kordia turn out to be the cultivar with the lowest pH value (3.10) and for the majority of cultivars pH value did not exceed 3.50. Only for four cultivars pH value were significantly higher 3.80-3.92 (Orleans, Regina, Trebnitzer Lotkirche and Schneiders Spate, respectively). Our

Table 1. pH, sugar, organic acid and anthocyanin content of the sweet cherries

Sweet cherry cultivars	pH	Sugars TSS (%)	Acidity (malic acid g 100 g^{-1} FW)	Total anthocyanin CRE (mg 100 g^{-1} FW)
Bianca*	3.30 ab	20.9 k	0.72 h	108. 5 i
Burlat	3.35 ab	14.3 a	0.43 a	36.0 cd
Johanna 1	3.25 ab	18.8 g	0.58 e	32.4 bcd
Johanna 2	3.20 ab	18.2 f	0.57 e	49.3 f
Kordia	3.10 a	18.2 f	0.56 e	46.8 ef
Kunzes Kirsche	3.25 ab	17.1 e	0.50 c	1.8 a
Merton Premier	3.25 ab	18.3 f	0.67 g	30.3 bc
Napoleon	3.46 b	19.4 i	0.76 i	1.5 a
Orleans	3.80 c	15.0 b	0.45 a	53.3 f
Regina	3.88 c	19.9 j	0.64 f	60.0 g
Rivan	3.20 ab	15.9 d	0.47 b	39.9 de
Schneiders Spate	3.92 c	15.7 c	0.46 b	64.4 g
Summit	3.45 b	19.9 j	0.53 d	28.1b
Trebnitzer Lot.	3.89 c	19.2 h	0.67 g	78.3 h

*Values followed by the same letter do not differ at $p < 0.05$

results for majority of cultivars indicate lower active acidity than in other studies. Reported are values of pH in the range from 3.56 to 3.80 which indicate also less variation between 12 cultivars (Hayaloglu and Demir 2015). They found for Summit cultivar pH 3.67 and in our study the value was as a rule lower 3.45. Higher pH values were found by other authors 4.0 to 4.4 (González-Gómez et al. 2010, Serradilla et al 2012).

Sugars are the main organic compound in sweet cherry, they gave specific taste sensations when eating fruit and affect consumer acceptance (Crisostoso et al. 2003, Usenik et al. 2008). Total soluble solids (TSS) dominant chemical component are sugars (90%). General evaluation of sugar content is performed by TSS measurement, accurate, simple and reproducible method (Girard and Kopp 1998). Significant variations were found in the total soluble solids content among cultivars (Tab. 1). TSS values were in the range of 14.3% to 20.9% for Burlat and Bianca cultivars respectively. Majority of cultivars were found to have TSS above 16%. Our results compared to the data available in the literature indicate that the determined sugar content of sweet cherry cultivars were slightly higher to the sweet cherries grown in other countries (Usenik et al. 2008, Kelebek and Selli 2011, Hayaloglu and Demir 2015). For Spanish Picota and Sweetheart cultivars similar results have been reported 14.0 and 23.3% respectively (Seradilla et al. 2012). Evaluation of 12 cultivars including Van, Summit and Sweetheart revealed TSS content 13.5 to 25.5% (González-Gómez et al. 2010). It is worth to point out that in both studies 'Sweetheart' TSS were the highest among other sweet cherries and the values were very close 23.3 and 25.5%. Such a similarity is not common within one specific cultivar. For example it was not true for 'Summit' – our presented results TSS 19.9% against 13.3% (Hayaloglu and Demir 2015). Differences indicate a possible effect of climatic conditions and cultural factors on the sugar content of fruit.

Organic acids are the second main group of organic compounds found in sweet cherries after carbohydrates. There are important components of sweet cherries in terms of their impact on the flavour (Usenik et al. 2008). Titratable acidity (Tab. 1) expressed as malic acid values ranged between 0.43 g ('Burlat') and 0.76 100 g^{-1} FW ('Napoleon'). For the cultivars studied by Hayaloglu and Demir (2015) in general the higher range of values was found as 0.71 g 100 g^{-1} FW ('Summit') to 1.01 ('Sweetheart'). For cultivar Summit evaluated also in our study acidity was slightly lower by close to 0.20 g 100 g^{-1} FW. Other reports present values in the range similar to previously cited study (Serrano et al. 2005, Serradilla et al. 2012). Much higher range of acidity 1.2 to 1.4 for sweet cherry fruit is also presented in literature (Kelebek and Selli 2011).

Anthocyanin content values were highly specific for cultivars and ranged from 108.5 ('Bianca') to 1.5 and 1.8 mg 100 g^{-1} FW ('Napoleon' and 'Kunzes Kirsche'), respectively. Obtained data

Table 2. Phenolic composition of sweet cherries (mg 100 g^{-1} FW)

Sweet cherry cultivars	Total phenolic CHAE	Tartaric esters CAE	Flawonoids QE
Bianca*	557.6 i	66.5 h	49.1 k
Burlat	162.2 cd	30.9 bc	21.3 fg
Johanna 1	151.9 bc	30.5 b	40.2 j
Johanna 2	194.4 f	36.3 ef	36.2 i
Kordia	176.8 de	30.2 b	25.1 h
Kunzes Kirsche	-	-	12.7 b
Merton Premier	146.7 bc	34.0 de	20.6 ef
Napoleon	101.1 a	31.5 bcd	15.3 c
Orleans	186.8 ef	30.1 b	17.1 d
Regina	237.6 g	37.7 f	19.5 e
Rivan	185.7 ef	38.5 f	20.1 ef
Schneiders Spate	235.6 g	33.7 cde	19.9 e
Summit	139.4 b	26.2 a	7.9 a
Trebnitzer Lot.	378.7 h	47.4 g	22.0 g

*Values followed by the same letter do not differ at $p < 0.05$

were higher than values reported by Hayaloglu and Demir (2015). One exception was Summit cultivar with total anthocyanin value 22.8 mg 100 g^{-1} FW CRE which was very close to 28.1 in our study. Our results in general are close in line with earlier reports in which total amounts of anthocyanins were 142 to 47 mg 100 g^{-1} of FW (Kelebek and Selli 2011). Gao and Mazza (1995) reported that the total anthocyanin contents is related to intensity of fruit colour. Anthocyanin contents could range from 82 to 297 mg 100 g^{-1} for dark sweet cherries and from 2 to 41 mg 100 g^{-1} for the light coloured ones. Our results support those findings.

The total content of phenolic in sweet cherries was significantly different among tested cultivars (Tab. 2). It ranged from 101 ('Napoleon') to 558 mg 100 g^{-1} FW CHAE ('Bianca'). In general total phenolic in studied cultivars were more abundant in relation to data given in literature. Gonçalves et al. (2004) reported that the total phenolic content in sweet cherry cultivars ranged from 6 to 230 mg 100 g^{-1} FW. A low range of values as compared to our study presented also other authors – 89 to 240 mg 100 g^{-1} of FW (Kelebek and Selli 2011).

The highest total tartaric esters content (Tab. 2) was detected in Bianca cultivar fruits followed by Trebnitzer Lotkirche and the lowest in Summit (66.5, 47.4, and 26.2 CAE mg 100 g^{-1} FW, respectively). The span of recorded values was one of the lowest among studied phenolic compounds.

Flavonoid contents of studied sweet cherries are shown in Table 2. There were significant differences between the cultivars. The highest content was detected in Bianca cultivar (49.1 QE mg 100 g^{-1} FW) and the lowest in 'Summit' (7.9 QE mg 100 g^{-1} FW). Our results are similar to obtained by Usenik et al. (2008) while in general lower than the values obtained by Gonçalves et al. (2004).

Antioxidant capacity of phenolic compounds was measured by two different methods: ABTS and DPPH assays. The ABTS assay is based on the generation of a blue/green ABTS, which is applicable to both hydrophilic and lipophilic antioxidant systems; whereas DPPH assay uses a radical dissolved inorganic media and is, therefore, applicable to hydrophobic systems (Kim et al. 2002). Table 3 presents the results of the antioxidant activities obtained by the studied cherry cultivars. DPPH scavenging method has been used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedure. Values varied significantly among sweet cherry cultivars. The highest and lowest DPPH radical scavenging activity after 30 minutes were observed in 'Bianca' (90%) and 'Kunzes Kirsche' (13%), respectively. Majority of values were in the range of 40 up to 59%. 'Bianca' fruit scavenging activity after only 5 minutes was very high – 74% in relation to other sweet cherries where values more than twice as low were recorded. Much lower variation of DPPH values among studied cultivars were obtained by Hayaloglu and Demir (2015). In general, the darker fruits in our study have higher antioxidant levels. The same results were

Table 3. Free radicals scavenging activity of sweet cherries (% of scavenging after 5 and 30 minutes)

Sweet cherry cultivars	DPPH		ABTS	
	5’	30’	5’	30’
Bianca*	74 j	90 i	70 g	88 g
Burlat	25 de	40 cd	24 bc	33 bc
Johanna 1	23 cd	37 c	24 c	35 cd
Johanna 2	29 g	47 f	28 de	39 de
Kordia	24 d	43 de	29 de	39 de
Kunzes Kirsche	10 a	13 a	18 a	26 a
Merton Premier	18 b	28 ab	20 ab	29 a
Napoleon	16 a	28 ab	21 ab	31 abc
Orleans	29 fg	46 ef	26 cd	35 bc
Regina	33 h	56 gh	31 e	43 e
Rivan	27 ef	43 e	30 e	41 e
Schneiders Spate	38 i	59 h	42 f	58 f
Summit	22 c	34 b	19 a	31 ab
Trebnitzer Lot.	35 h	53 g	32 e	54 f

*Values followed by the same letter do not differ at $p < 0.05$

observed by Gonzales-Gomez et al. (2010), Kaur and Kapoor (2001). Scavenging activity of for highest dark fruits according to our data were twice as much as lowest one. Kelebek and Selli (2011) reported similar results for 4 sweet cherry cultivars and Serradilla et al. (2011), Usenik et al. (2008) for 13 cultivars.

ABTS method is commonly applied to measure antioxidant activity in food. ABTS values of sweet cherry samples changed between 88 and 26% (after 30 minutes). The highest antioxidant capacity was measured in ‘Bianca’ and the lowest in ‘Kunzes Kirsche’ respectively. Majority of values were in the range of 30 up to 40%. ‘Bianca’ fruit scavenging activity after only 5 minutes was very high – 70%. It is worth to point out that the same order of cultivars based on data for ABTS was recorded by DPPH assay. Both methods produced quite similar values suggesting strong correlations among them. Significant correlations between ABTS and DPPH were found in other studies (Dudonné et al. 2009, Kelebek and Selli 2011). In general, the darker fruits have higher antioxidant capacity as evaluated by ABTS method. The same results were observed by Gozales-Gomez et al. (2010) and Liu et al. (2011).

The total phenolic contents and antioxidant capacities applying ABTS and DPPH methods were compared (Tab. 4). Our results show strong correlations between antioxidative capacity and total phenolics content (ranging from 0.908 to 0.982, $p < 0.05$). Our results indicate stronger correlations then in the results of Kelebek and Selli (2011), who reported the values 0.811 and 0.741, respectively. Those authors found also that anthocyanins exhibited strong positive correlations with antioxidant activity assays (0.930 to 0.951). Similar conclusion can be drawn based on our results.

Table 4. Correlation coefficients between antioxidant capacity measured by ABTS and DPPH and phenolic content of sweet cherries

	ABTS	DPPH
Anthocyanins	0.834	0.970
Flavonols	0.914	0.974
Tartaric esters	0.907	0.972
Total phenolics	0.908	0.982
ABTS	1	0.876
DPPH	0.876	1

Correlations between antioxidant capacity by ABTS and DPPH assay revealed significant positive value (0.876). Our results are in agreement with other studies, however reported were stronger positive correlations between ABTS and DPPH. The values were 0.906 and 944 respectively (Dudonne et al. 2009, Kelebek and Selli 2011).

CONCLUSIONS

To the best of our knowledge this is the 1-st study on chemical composition of most sweet cherry cultivars grown in Poland. The results showed significant influence of cultivars on studied compounds – sugars, organic acids, phenolic

contents, and antioxidant activity. The highest sum of total sugars expressed as TSS was found in 'Bianca' and the lowest in 'Burlat'. Organic acid content was the lowest in 'Burlat' and the highest in 'Napoleon'. Anthocyanin content was the highest in 'Bianca' and the lowest in 'Napoleon' and 'Kunzes Kirsche'. Total phenolics, anthocyanin, phenolic acids, and flavonoids contents, antioxidant activity as measured by ABTS and DPPH were highly specific for each cultivar. In particular, 'Bianca' was characterized by the highest values of total phenolic and anthocyanin contents as well as tartaric esters and flavonoids. 'Bianca' has also the highest scavenging activity assayed by ABTS and DPPH. Very strong correlations between antioxidant capacity and phenolic contents were found, indicating that phenolic compounds are the major contributors to the antioxidant properties of sweet cherries.

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AUTHOR CONTRIBUTIONS

J.S. designed experiment and generate the main idea of article, M.L. was very inspiring in discussions, collect a lot of literature, analytical measurements analysis. A.G. statistical elaboration of data. J.S., M. L. and A.G. equally contributed in manuscript writing and final state of the paper. P.B. was responsible for the maintenance of the experimental orchard and served us with his knowledge and experience in estimation of harvest date for each of the cultivars.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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