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Effects of Rootstock and Training System on Tree Canopy, Fruit Quality and Phytochemicals of '0900 Ziraat' and 'Regina' Sweet Cherry Cultivars

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HIGHLIGHTS

- The rootstock had a significant effect on the vegetative growth
- With the 0900 Ziraat x Krymsk 5 combination, the trees formed thicker TCSA
- The Regina x Piku 1 combination created the trees with a larger canopy
- The cultivar has affected fruit quality and bioactive compound content.

ABSTRACT: Both '0900 Ziraat' and 'Regina' grafted on 'Krymsk 5', or 'Piku 1' rootstocks were trained to either Upright Fruiting Offshoots (UFO), Super Slender Axe (SSA) or Kym Green Bush (KGB) training systems. Vegetative growth of the tree, determined by measuring trunk cross-sectional area (TCSA), canopy volume and leaf area, differed significantly, depending on the cultivar x rootstock x training system combination. In general, 'Krymsk 5' rootstock resulted in trees with significantly thicker trunks (TCSA: 37.75 cm²) and increased leaf area (up to 86.97 cm²). Fruit weight and fruit quality parameters including Hunter a*, firmness, TSS and acidity were variable between rootstocks and training systems and often not significantly different between treatments. In some years however, significant differences were highly dependent on the training system and rootstock interactions. Higher concentrations of bioactive phytochemical concentrations for total monomeric anthocyanin and antioxidant concentrations were mostly associated with the UFO training system in conjunction with the 'Krymsk 5' rootstock suggesting that these are linked to increased tree vigour and increased leaf surface area.

Keywords: canopy management; *Prunus avium*; health.

INTRODUCTION

Sweet cherry trees grafted onto seedling rootstocks produce large trees with strong vegetative growth, narrow-angled branches, and apical dominance such trees lack precocity due to an extended juvenile phase, resulting in low yields and poor fruit quality during the first 5-6 years after planting [1]. Excessively large trees are difficult to spray, prune and harvest. Sweet cherry breeding programmes have focused on tree size control by introducing semi-dwarfing rootstocks. While the choice of the rootstock effectively controls tree size, selecting the appropriate training system for the rootstock x cultivar combination is crucial as major differences have been observed over multiple years [2] and self-fertile cultivars behave differently than non-self-fertile cultivars [3]. Previous studies on sweet cherries found that yield [4, 5] and fruit quality [5-10] differed significantly between different scion x rootstock combinations vigour [11,12]. Additional studies found that rootstocks also affect tree vigour [13], as well as biochemical composition and phytochemical concentrations of fruit [9, 14].

Training systems affect development, position and angle of the branches, and thus light interception, which in turn affects yield and fruit quality [15]. Training systems simplify tree architecture, enabling efficient use of the orchard area, increased light interception and even distribution of that light over the entire canopy leaf area [16]. Furthermore, training system may help regulate tree structure, increasing formation of flower buds and reducing negative effects of the shading on fruit development [17]. Training systems should also be selected according to planting density and regional adaptation [16]. Different scion x rootstock combinations result in different responses depending on type of pruning cuts, and time of pruning [18,19].

Many previous studies considered effects of rootstocks on different training systems but few considered effects of rootstock x cultivar x training system and certainly not in Türkiye. Consequently, the current study examined effects of two different semi-dwarfing rootstocks x three different training systems on the performance of 'Regina' and '0900 Ziraat' sweet cherry trees.

MATERIALS AND METHODS

Sweet cherry trees of '0900 Ziraat' and 'Regina' were grafted onto 'either 'Krymsk 5' or 'Piku 1' semi-dwarfing rootstocks and planted in (2017) at Sezai Karakoç Vocational and Technical Anatolian High School (40° 10 '21.77 "North, 38° 06' 02.34" East and altitude 972 m) in Susehri district of Sivas province under drip irrigation and maintained according to commercial orchard practices including annual fertilization as well as pest, disease and weed control. Trees were planted in 2016 and trained to three different training systems, namely Kym Green Bush (KGB) (4m x 1m), Super Slender Axe (SSA) (4m x 2m) or Upright Fruiting Offshoots (UFO) (4m x 2m) training systems. The study was designed as a split-plot design with four replications, and there were six trees in each replication. Vegetative growth was recorded in 2018 and 2019, while the fruit quality characteristics were determined for fruit harvested in 2019 and 2020. However, due to lack of precocity in the KGB training system, fruit set did not occur on the trees trained to that system. So fruit quality characteristics had not been evaluated for this training system.

Trunk diameter (cm) was recorded at a height of 15 cm above the graft union with a (Need the brand name, manufacturer and place of manufacture) digital calliper with a sensitivity of ± 0.01 mm. Trunk cross-sectional area (cm^2) was calculated by using the formula $TCSA = \pi \cdot r^2$. Two measurements (m) were taken of the north-south and east-west directions in the middle of each tree canopy and the results were averaged for each tree. Canopy height (m) was recorded by measuring the distance between the point where the lowest branch occurred and the top of the canopy. Canopy volume was calculated using the formula $V = \pi r^2 \cdot h / 2$ and expressed in m^3 . In July of each growth period, 30 leaves from each tree were randomly collected from annual shoots) and measured by a digital leaf area meter (LI-COR, Bioscience, USA) and expressed in cm^2 .

Fruit quality characteristics

At harvest, 20 fruit were randomly selected from each tree. Mass was recorded using a Brandname digital scale (0-5000 g \pm 0.01 g). (Radwag, Poland) as well as fruit colour using a Minolta™ CR-400 colourimeter (Konica Minolta Inc., Japan) Colourimeter measurements were recorded at opposite points of the equatorial part of each fruit and averaged. Fruit colour was determined as a * value. Fruit firmness for each fruit was measured using a Durofel digital firmness meter (Agrosta Instruments, Agrotechnologie, France). The 10 mm end of the device was brought into contact with the opposite cheeks of the equatorial part of the fruit vertically. The scale ranges from 0 to 100 for very soft to very firm surfaces [20]. Twenty fruits from each tree were juiced, from which total soluble solids concentration was recorded using a hand-held Atago PAL-1 Digital refractometer Atago Co. Ltd., Tokyo, Japan). Titratable acidity was also measured by titration with 0.1 N NaOH and expressed in g malic acid 100 ml of juice using a pH meter.

Phytochemical concentrations

Vitamin C concentration was determined using a Merck RQflex® 20 Reflectoquant® (Merck Millipore, Massachusetts, USA) A 0.5 ml aliquot of fruit juice was added to 4.5 ml of 0.5% oxalic acid subsequently, an ascorbic acid test kit (Catalog no: 116981, Merck, Germany) was immersed in the solution for 2 seconds, then held at ambient temperature for 8 sec to allow the test kit to oxidize and then read in the Reflectoquant device after an additional 7^h sec. Results were expressed as mg of vitamin C.100 g⁻¹ [21].

Total phenolics, antioxidant capacity, and monomeric anthocyanin were measured for 30 randomly selected fruit per tree for replicate. Stones and fruit flesh were separated and fruit flesh was homogenized using a blender and approximately 100 g of the fruit flesh was stored in a deep freezer at -20 °C in falcon tubes for analysis at a later date.

Total phenolics were determined using Folin-Ciocalteu reagent as described in the study of [22]. Fruit extract, Folin-Ciocalteu and distilled water were mixed in a 1: 1: 20 and then 7% sodium carbonate was added. Following two hours of incubation, the solution turned a bluish colour, and was measured in the spectrophotometer at 750 nm wavelength. Results were expressed as µg gallic acid equivalent (GAE) g⁻¹ fresh fruit (fw).

Total monomeric anthocyanin concentration in the fruit was determined using the pH difference method [23]. Extracts were prepared at pH 1.0 and 4.5 and measured at 520 and 700 nm wavelengths. Total monomeric anthocyanin amount (molar extinction coefficient of 29600 cyanidin-3-glucoside) was determined as absorbances [(A520 - A700) pH 1.0 - (A520 - A700) pH 4.5] and expressed as µg cyanidin-3-glucosides (cy-3-glu) g⁻¹ fresh weight (fw).

Antioxidant concentrations were determined using Trolox Equivalent Antioxidant Capacity (TEAC) method [24]. Here, 7 mM ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) was mixed with 2.45 mM potassium bisulfate and kept in the dark for 12-16 hours, after which absorbance of 0.700 ± 0.01 mL at a wavelength of 734 nm in the spectrophotometer using sodium acetate (pH 4.5). Finally, by mixing 2.98 mL of prepared buffer into 20 µL of fruit extract, absorbance was measured using a spectrophotometer at 734 nm wavelength after 10 minutes. Absorbance values obtained were calculated with Trolox (10–100 µmol / L) standard slope chart and expressed as µmol Trolox Equivalent Antioxidant Capacity (TEAC) g⁻¹ fresh weight (fw).

Statistical analysis

Data were analysed by General Analysis of Variance and differences between means were determined using the Tukey multiple comparison test. Statistical analyses were performed using the SAS Ver. 9. (SAS Institute Inc., North Carolina, USA). Statistical significance is reported at P = 5%.

RESULTS

Trunk cross-sectional area (TCSA), canopy volume and leaf area

In general, both '0900 Ziraat' and 'Regina' scion cultivars formed thicker trunks on 'Krymsk 5' than 'Piku 1', regardless of the training system (Table 1). In 2018, '0900 Ziraat' on 'Krymsk 5' rootstocks trained to KGB had the thickest trunks (TCSA = 19.44 cm²) and only '0900 Ziraat' on 'Krymsk 5' trained to UFO was not significantly different (TCSA = 17.5 cm²) from this. All other rootstock x scion x training system combinations had significantly smaller trunks. Trees with the smallest trunks were observed in '0900 Ziraat' on 'Piku 1' trained to SSA (TCSA = 13.15 cm²) but this was not significantly different from '0900 Ziraat' on 'Piku 1' trained to the UFO (TCSA = 14.12 cm²) or 'Regina' on 'Piku 1' trained to KGB (TCSA = 14.13 cm²) or SSA (TCSA = 13.27 cm²) not 'Regina' on 'Krymsk 5' trained to SSA (TCSA = 14.42 cm²).

In 2019, the pattern was repeated with trunks of both scions having significantly thicker trunks on 'Krymsk 5' rootstocks than 'Piku 1' rootstocks (Table 1). Again, '0900 Ziraat' on 'Krymsk 5' trained to KGB (TCSA = 37.75 cm²) and UFO (TCSA = 31.71 cm²) had significantly thicker trunks than all other rootstock x scion combinations. Furthermore, 'Regina' on 'Piku 1' trained to SSA had the smallest trunks (TCSA = 18.22 cm²). These were not significantly different from trunks of 'Regina' on 'Piku 1' trained to UFO (TCSA = 18.28 cm²) or '0900 Ziraat' on 'Piku 1' trained to SSA (TCSA = 20.47 cm²).

Table 1. Effects of rootstocks and training systems on trunk cross-sectional area (TCSA) of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	TCSA (cm ²)		
		2018		
		KGB	UFO	SSA
'0900 Ziraat'	'Piku 1'	15.56 bc	14.12 cd	13.15 d
	'Krymsk 5'	19.44 a	17.50 ab	16.10 b
'Regina'	'Piku 1'	14.13 cd	16.38 b	13.27 d
	'Krymsk 5'	17.23 ab	16.35 b	14.42 cd
2019				
'0900 Ziraat'	'Piku 1'	21.47 C	23.92 BC	20.47 CD
	'Krymsk 5'	37.75 A	31.71 A	26.99 B
'Regina'	'Piku 1'	24.70 BC	18.28 D	18.22 D
	'Krymsk 5'	29.07 AB	26.01 B	27.49 B

KGB: Kym Green Bush. UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences in 2018 at P<0.05. Upper case letters indicate significant differences in 2019 at P<0.05.

In 2018, canopy volumes of all rootstock × cultivar × training system combinations were similar to each other (Table 2). In 2019, however, 'Regina' on 'Krymsk 5' trained to SSA had the largest canopy volume (4.63 m³) but this was only significantly bigger than 'Regina' on 'Piku 1' trained to either a UFO (1.35 m³) or KGB (2.10 m³) or 'Regina' on 'Piku 1' trained to UFO (1.93 m³) (Table 2). It is interesting that the trees with the thickest trunks in 2019, namely '0900 Ziraat' on 'Krymsk 5' trained to KGB did not have the biggest canopies (3.69 m³) but it should be noted that this was not significantly different from the trees with the biggest canopies ('Regina' on 'Krymsk 5' trained to SSA had the largest canopy volume (4.63 m³).

Table 2. Effects of rootstocks and training systems on average canopy volume of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Canopy volume (m ³)		
		2018		
		KGB	UFO	SSA
'0900 Ziraat'	'Piku 1'	1.41 a	0.94 a	1.62 a
	'Krymsk 5'	2.11 a	1.94 a	3.43 a
'Regina'	'Piku 1'	1.33 a	1.05 a	2.37 a
	'Krymsk 5'	2.36 a	1.88 a	3.37 a
2019				
'0900 Ziraat'	'Piku 1'	2.10 B	1.35 B	2.16 AB
	'Krymsk 5'	3.69 A	2.49 AB	4.05 A
'Regina'	'Piku 1'	3.09 A	1.93 B	2.21 AB
	'Krymsk 5'	3.83 A	2.55 AB	4.63 A

KGB: Kym Green Bush. UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2018 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05.

In 2018, leaf area of both scion × rootstock by all training system combinations were not significantly different (Table 3). In 2019, however, leaf area was significantly smaller in 'Regina' on 'Piku 1' trained to SSA (53.57 cm²), '0900 Ziraat' on 'Piku 1' trained to both UFO (62.58 cm²) and SSA (64.10 cm²) respectively, compared to all other scion × rootstock combinations. By comparison, '0900 Ziraat' on 'Krymsk 5' trained to SSA had the highest leaf area (86.97 cm²) (Table 3).

Table 3. Effects of rootstocks and training systems on leaf area of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Leaf area (cm ²)		
		2018		
		KGB	UFO	SSA
'0900 Ziraat'	'Piku 1'	70.90 a	76.48 a	81.38 a
	'Krymsk 5'	83.86 a	83.95 a	82.81 a
'Regina'	'Piku 1'	69.93 a	69.51 a	67.80 a
	'Krymsk 5'	75.97 a	73.47 a	79.13 a
2019				
'0900 Ziraat'	'Piku 1'	75.37 A	62.58 B	64.10 B
	'Krymsk 5'	69.70 A	70.25 A	86.97 A
'Regina'	'Piku 1'	73.60 A	73.42 A	53.57 B
	'Krymsk 5'	71.13 A	78.83 A	71.60 A

KGB: Kym Green Bush. UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2018 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05.

Fruit weight, Hunter a* values and fruit firmness

In 2019, '0900 Ziraat' on 'Piku 1' trained to SSA resulted in the largest fruit on average (7.79 g). This was, however, only significantly larger on average than fruit from '0900 Ziraat' on 'Krymsk 5' trained to SSA (5.78 g), 'Regina' on 'Krymsk 5' on SSA (5.70 g) or 'Regina' on 'Piku 1' trained to UFO (5.76 g). All other scion x rootstock x training system produced fruit of similar size (Table 4). In 2020, all fruit for all scion x rootstock x training system combinations were similar in size with no significant differences being observed.

Table 4. Effects of rootstocks and training systems on average fruit weight of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Fruit weight (g)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	7.62 a	7.79 a
	'Krymsk 5'	6.85 a	5.78 b
'Regina'	'Piku 1'	5.76 b	6.19 a
	'Krymsk 5'	6.47 a	5.70 b
2020			
'0900 Ziraat'	'Piku 1'	7.13 A	7.06 A
	'Krymsk 5'	7.39 A	7.13 A
'Regina'	'Piku 1'	5.73 A	5.88 A
	'Krymsk 5'	5.60 A	6.01 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2020 at P<0.05.

In 2019, a* values, which are a direct measure of red skin colour of the fruit, were not significantly different for any of the scion x rootstock x training system combinations evaluated (Table 5). In 2020, however, Hunter a* values of fruit from '0900 Ziraat' on 'Krymsk 5' trained to UFO (23.48) and '0900 Ziraat' on 'Piku 1' trained to SSA (24.38) and 'Regina' on identified fruit with the least red skins. Red skin colour of these fruit were not significantly different from '0900 Ziraat' on 'Piku 1' trained to UFO (25.09) but all other scion x rootstock x training system combinations resulted in fruit with redder skins.

Table 5. Effects of rootstocks and training systems on Hunter a* value of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Hunter a*	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	30.59 a	29.74 a
	'Krymsk 5'	32.00 a	33.36 a
'Regina'	'Piku 1'	34.14 a	32.07 a
	'Krymsk 5'	32.84 a	33.18 a
2020			
'0900 Ziraat'	'Piku 1'	25.09 AB	24.38 B
	'Krymsk 5'	23.48 B	32.88 A
'Regina'	'Piku 1'	31.20 A	27.44 A
	'Krymsk 5'	34.27 A	31.64 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2020 at P<0.05.

In 2019, fruit from '0900 Ziraat' on 'Piku 1' trained to UFO were the firmest (44.80) but these were not significantly different than those from 'Regina' on 'Piku 1' trained to a UFO (40.93) (Table 6). Fruit from 'Regina' on both 'Krymsk 5' and 'Piku 1' trained to SSA (27.03 and 27.97 respectively) and '0900 Ziraat' on 'Krymsk 5' trained to SSA (28.47) were significantly softer than all other scion x rootstock x training system combination. In 2020, all fruit from both '0900 Ziraat' and 'Regina' trees grafted on 'Krymsk 5' produced significantly firmer fruit than those grafted on 'Piku 1' for both UFO and SSA training systems. The firmest fruit on average were 'Regina' on 'Krymsk 5' trained to UFO (80.45) and the softest fruit were '0900 Ziraat' on 'Krymsk 5' trained to SSA (50.63) (Table 6).

Table 6. Effects of rootstocks and training systems on fruit firmness (Scale 0-100 where 0 is very soft and 100 is very firm) of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Fruit firmness *	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	44.80 a	38.03 b
	'Krymsk 5'	35.89 b	28.47 c
'Regina'	'Piku 1'	40.93 a	27.97 c
	'Krymsk 5'	34.98 b	27.03 c
2020			
'0900 Ziraat'	'Piku 1'	57.77 B	50.63 B
	'Krymsk 5'	71.32 A	68.45 A
'Regina'	'Piku 1'	62.15 B	58.58 B
	'Krymsk 5'	80.45 A	73.73 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2020 at P<0.05.

Total Soluble Solids concentration (TSS), titratable acidity and vitamin C

In 2019, 'Regina' on 'Piku 1' had significantly higher TSS (14.53%) than 'Regina' on 'Krymsk 5' (from 12.83 - 12.5%) regardless of the training system (Table 7). '0900 Ziraat' however only had significantly higher TSS on 'Piku 1' when trained to the SSA (14.37%). In 2020, the SSA training system produced fruit with higher TSS (up to 17.10%) when compared to UFO for all scion x rootstock combinations, with the exception

of '0900 Ziraat' on 'Krymsk 5' trained to UFO (16.70%). The latter was not significantly different from fruit harvested from all trees trained to the SSA system.

Table 7. Effects of rootstocks and training systems on TSS (%) of '0900 Ziraat' and 'Regina' sweet cherries.

Cultivars	Rootstock	TSS (%)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	13.20 a	14.37 a
	'Krymsk 5'	13.93 a	12.57 b
'Regina'	'Piku 1'	14.53 a	14.53 a
	'Krymsk 5'	12.83 b	12.50 b
2020			
'0900 Ziraat'	'Piku 1'	16.20 B	16.67 A
	'Krymsk 5'	16.70 A	16.30 A
'Regina'	'Piku 1'	15.97 B	17.10 A
	'Krymsk 5'	15.47 B	16.40 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at $P < 0.05$. Uppercase letters indicate significant differences determined by Tukey test in 2020 at $P < 0.05$.

Acidity was extremely variable with no clear training system influence on either scion cultivar (Table 8). In 2019, fruit from 'Regina' on 'Krymsk 5' had significantly lower acidity than those on 'Piku 1'. Again, in 2020, acidity was extremely variable with only '0900 Ziraat' on 'Krymsk 5' having higher acidity ($1.01 \text{ g malic acid} \cdot 100 \text{ mL}^{-1}$) than all other '0900 Ziraat' rootstock x scion combinations. 'Regina' on 'Piku 1' trained to UFO and Regina on 'Krymsk 5' trained to SSA were significantly higher ($0.99 \text{ g malic acid} \cdot 100 \text{ mL}^{-1}$) than their direct comparisons.

Table 8. Effects of rootstocks and training systems on acidity of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Acidity ($\text{g malic acid} \cdot 100 \text{ mL}^{-1}$ juice)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	0.51 b	0.61 a
	'Krymsk 5'	0.58 a	0.56 b
'Regina'	'Piku 1'	0.62 a	0.62 a
	'Krymsk 5'	0.54 b	0.56 b
2020			
'0900 Ziraat'	'Piku 1'	0.90 B	0.96 B
	'Krymsk 5'	0.96 B	1.01 A
'Regina'	'Piku 1'	0.99 A	0.92 B
	'Krymsk 5'	0.95 B	0.99 A

KGB: Kym Green Bush. UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at $P < 0.05$. Uppercase letters indicate significant differences determined by Tukey test in 2020 at $P < 0.05$.

In 2019, Vitamin C concentrations of fruit from '0900 Ziraat' on 'Piku 1' trained to UFO were significantly lower ($5.85 \text{ mg} \cdot 100\text{g}^{-1}$) than all other '0900 Ziraat' rootstock x scion combinations. 'Regina' on 'Piku 1' trained to the UFO also resulted in higher Vitamin C concentrations ($8.15 \text{ mg} \cdot 100\text{g}^{-1}$) than 'Regina' on 'Piku 1' trained to the SSA ($6.05 \text{ mg} \cdot 100\text{g}^{-1}$) but was not significantly different than those from 'Regina' on 'Krymsk 5' regardless of training system. In 2020, Vitamin C concentrations of 'Regina' fruit on all rootstock x training

systems were significantly higher (up to 8.95 mg.100g⁻¹) than all '0900 Ziraat' rootstock x training systems (ranging from 5.65 to 6.30 mg.100g⁻¹) with the exception of '0900 Ziraat' on 'Piku 1' trained to SSA (7.90 mg.100g⁻¹) (Table 9).

Table 9. Effects of rootstocks and training systems on vitamin C concentration (mg.100 g⁻¹ fresh weight (fw)) of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Vitamin C (mg.100 g ⁻¹ fw)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	5.85 b	6.80 a
	'Krymsk 5'	7.80 a	7.20 a
'Regina'	'Piku 1'	8.15 a	6.05 b
	'Krymsk 5'	7.60 a	7.00 a
2020			
'0900 Ziraat'	'Piku 1'	5.65 B	7.90 A
	'Krymsk 5'	6.30 B	6.20 B
'Regina'	'Piku 1'	8.45 A	8.95 A
	'Krymsk 5'	7.75 A	7.85 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2020 at P<0.05.

Total phenolics, total monomeric anthocyanin and antioxidant capacity

In 2019, total phenolic concentrations (Table 10) of the fruit from '0900 Ziraat' on 'Piku 1' were significantly higher (1265 to 1276 ug gallic acid equivalent g⁻¹ fresh weight) than those from '0900 Ziraat' on 'Krymsk 5' trained to UFO (1048 ug gallic acid equivalent g⁻¹ fresh weight). In addition, 'Regina' on 'Piku1' trained to UFO were significantly higher (1259 ug gallic acid equivalent g⁻¹ fresh weight) than 'Regina' on 'Krymsk 5' regardless of training system (1030-1053 ug gallic acid equivalent.g⁻¹ fresh weight). In 2020, there were no significant differences within either '0900 Ziraat' or 'Regina' regardless of either rootstock or training system. Only 'Regina' on 'Piku 1' trained to UFO (967 ug gallic acid equivalent.g⁻¹ fresh weight) and 'Regina' on 'Krymsk 5' trained to SSA (837 ug gallic acid equivalent.g⁻¹ fresh weight) were significantly higher than '0900 Ziraat' on 'Piku 1' regardless of training system (ranging from 578-661 ug gallic acid equivalent.g⁻¹ fresh weight) or '0900 Ziraat' on 'Krymsk 5' trained to UFO (655 ug gallic acid equivalent.g⁻¹ fresh weight).

Table 10. Effects of rootstocks and training systems on total phenolics (µg gallic acid equivalent (GAE).g⁻¹ fresh weight (fw)) of '0900 Ziraat' and 'Regina' sweet cherry cultivars.

Scion	Rootstock	Total phenolics (µg GAE.g ⁻¹ fw)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	1276 a	1265 a
	'Krymsk 5'	1048 b	1144 ab
'Regina'	'Piku 1'	1259 a	1184 ab
	'Krymsk 5'	1053 b	1030 b
2020			
'0900 Ziraat'	'Piku 1'	578 B	661 B
	'Krymsk 5'	655 B	752 AB
'Regina'	'Piku 1'	967 A	778 AB
	'Krymsk 5'	798 AB	837 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at P<0.05. Uppercase letters indicate significant differences determined by Tukey test in 2020 at P<0.05.

In 2019, total monomeric anthocyanin concentrations (Table 11) of fruit was highest in both '0900 Ziraat' and 'Regina' on 'Krymsk 5' trained to UFO (40.44 and 36.86 µg cy-3-glu.g⁻¹ f.wt respectively). In 2020, the

SSA training system resulted in higher anthocyanin concentrations ($7.94 - 9.93 \mu\text{g cy-3-glu.g}^{-1} \text{ f.wt}$) than the UFO training system except for '0900 Ziraat' on Krymsk 5' ($3.49 \mu\text{g cy-3-glu.g}^{-1} \text{ f.wt}$).

Table 11. Effects of rootstocks and training systems on average total monomeric anthocyanin concentration ($\mu\text{g cyanidin-3-glucosides (cy-3-glu).g}^{-1} \text{ fresh weight (fw)}$) of '0900 Ziraat' and 'Regina' sweet cherries.

Scion	Rootstock	Total monomeric anthocyanin ($\mu\text{g cy-3-glu.g}^{-1} \text{ fw}$)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	28.38 b	19.20 b
	'Krymsk 5'	40.44 a	28.75 b
'Regina'	'Piku 1'	31.99 b	18.84 b
	'Krymsk 5'	36.86 a	26.37 b
2020			
'0900 Ziraat'	'Piku 1'	4.91 B	7.94 A
	'Krymsk 5'	4.25 B	3.49 B
'Regina'	'Piku 1'	4.45 B	9.51 A
	'Krymsk 5'	4.05 B	9.93 A

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at $P < 0.05$. Uppercase letters indicate significant differences determined by Tukey test in 2020 at $P < 0.05$.

In 2019, antioxidant concentrations (Table 12) of fruit from 'Regina' on both rootstocks were greater on trees trained to UFO (ranging from $3.63 - 3.68 \mu\text{mol TEAC.g}^{-1} \text{ fw}$) than SSA (ranging from $3.31 - 3.39 \mu\text{mol TEAC.g}^{-1} \text{ fw}$) and were similar to '0900 Ziraat' on 'Krymsk 5' trained to UFO ($3.97 \mu\text{mol TEAC.g}^{-1} \text{ fw}$) and '0900 Ziraat' on 'Piku 1' trained to SSA ($3.50 \mu\text{mol TEAC.g}^{-1} \text{ fw}$). In 2020, only fruit from 'Regina' on 'Piku 1' trained to SSA ($4.15 \mu\text{mol TEAC.g}^{-1} \text{ fw}$) were significantly higher than all other scion x rootstock x training system combinations (ranging from $2.10 - 2.81 \mu\text{mol TEAC.g}^{-1} \text{ fw}$), none of which were significantly different from each other.

Table 12. Effects of rootstocks and training systems on average antioxidant concentration ($\mu\text{mol Trolox Equivalent Antioxidant Capacity (TEAC).g}^{-1} \text{ fresh weight (fw)}$) of '0900 Ziraat' and 'Regina' sweet cherries

Scion	Rootstock	Antioxidant concentration ($\mu\text{mol TEAC.g}^{-1} \text{ fw}$)	
		2019	
		UFO	SSA
'0900 Ziraat'	'Piku 1'	3.21 b	3.50 a
	'Krymsk 5'	3.97 a	3.12 b
'Regina'	'Piku 1'	3.68 a	3.39 b
	'Krymsk 5'	3.63 a	3.31 b
2020			
'0900 Ziraat'	'Piku 1'	2.10 B	2.54 B
	'Krymsk 5'	2.24 B	2.11 B
'Regina'	'Piku 1'	2.58 B	4.15 A
	'Krymsk 5'	2.81 B	2.80 B

UFO: Upright Fruiting Offshoots. SSA: Super Slender Axe. Lowercase letters indicate significant differences determined by Tukey test in 2019 at $P < 0.05$. Uppercase letters indicate significant differences determined by Tukey test in 2020 at $P < 0.05$.

DISCUSSION

In sweet cherry trees, large, upright tree structures occur due to excessive vegetative vigour, which leads to strong apical dominance with a tendency to form narrow branch angles or pendant wood, which produces small, soft fruit. Both branching habits complicates cultural practices such as pruning and training and hinder

harvest, resulting in low yields of poor-quality fruit [2]. To alleviate this problem, ideal scion x rootstock x training system combinations must be identified for a given climate [4, 13] and will have a direct effect on tree growth and vigour [13, 25]. [3] also reported that different scion x rootstock x training systems perform differently in different regions.

The current study determined that TCSA, canopy volume and leaf area, differed depending on the scion x rootstock x training system combination. In general, 'Krymsk 5' rootstocks formed significantly thicker trunks than 'Piku 1' rootstocks and the KGB training system also resulted in significantly thicker trunks than the UFO and SSA systems. Given the fact that KGB tree trunks are pruned more heavily than the other two systems, it is expected that secondary thickening would lead to thicker trunks. The finding that KGB trees also had significantly more canopy in the last year of the study is further evidence for this. What was interesting was that both scion cultivars on 'Krymsk 5' trained to SSA also had similarly large canopy volumes and tree height, width and depth are implicated in this last finding. Similarly, others found that tree size in sweet cherry varies depending on the rootstock, while the effect of the rootstock x cultivar combination also plays a significant role [18, 19].

Adequate light distribution in the tree canopy is required for obtaining good fruit quality. Large dense, vertical tree structures limit light interception causing a decrease in fruit quality characteristics such as yield, fruit weight, colour, soluble solids content and acidity [26-28].

Enzymes and proteins of the sun-exposed leaves have higher activity and efficiency than shaded ones [29,30]. [31] emphasized that there are different light zones in the tree canopy and that the two most important factors affecting light interception are tree size and tree structure. Furthermore, there is a direct correlation between tree size and shaded unproductive leaf area, so the smaller the tree size, the smaller the percentage of unproductive leaves that receive the least light. Effects of scion x rootstock x pruning practices have been known to affect fruit quality characteristics for some time [32]. In the current study, the effects of the cultivar, rootstock and training system on quality characteristics such as fruit size, colour, fruit firmness, TSS and acidity were significant. However, differences were not always consistent between years of for that matter between different rootstock or scion combinations. In general, inconsistencies in fruit quality characteristics were more often due to rootstock differences but training systems were also implicated. Furthermore, these differences may become more pronounced as the trees age and reach full canopy. Similarly, previous studies have also highlighted the effect of the rootstock [7-10], cultivar [4,6] (and training system [13,25] on fruit quality characteristics in sweet cherry.

The concentration of bioactive phytochemical compounds of fruit may vary depending on ecological factors, rootstock [33], cultivar [34] and pruning [35]. In the current study, the effect of the rootstock x cultivar x training system had significant effects on concentrations of bioactive phytochemical compounds such as vitamin C, total phenolics, monomeric anthocyanin, and antioxidant capacity. In general, bioactive phytochemical concentrations were generally higher in combinations that had more vigorous growth. Indeed, [9] and [36] have reported that trees, which differed in vigour, had different concentrations of bioactive phytochemical compounds, while [37] reported that there was a positive correlation between the tree vigour and phenolic and flavonoids concentrations of fruit.

In general, 'Krymsk 5' resulted in thicker trunks with increased leaf area. In general, fruit quality characteristics were better and bioactive phytochemical concentrations was higher in combinations with vigorous growth.

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