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Nectar secretion and honey production potential of Satsuma mandarin (*Citrus unshiu* Marc.) cultivars

Izlučivanje nektara i medonosni potencijal unšijske mandarine (*Citrus unshiu* Marc.)

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ABSTRACT

This is the first reported study aimed at nectar secretion of Satsuma mandarin cultivars from Croatia. The study was conducted in 2014-2016 on four cultivars: Kawano Wase, Okitsu, Chahara and Zorica. Nectar was collected at six measurements per day at two-hour intervals (8:00-18:00) for a total of 12 days. The mean values of nectar quantity per flower were higher in Okitsu (23.41 µL) and Kawano Wase (22.79 µL) cultivars and were not statistically different ($P > 0.05$) from the other cultivars, while a significant difference ($P < 0.05$) was found in Chahara (21.60 µL) cultivar compared to Zorica (20.01 µL). Secreted nectar ranged from a minimum of 5.00 µL to a maximum of 75.00 µL among the studied cultivars. When comparing sugar concentration by cultivars, the lowest concentration was found in the cultivar Zorica (18.43%) and differed significantly ($P < 0.05$) from the others, while the mean values of the studied parameter in the cultivars Okitsu (20.35%), Kawano Wase (20.66%) and Chahara (20.17%) did not differ significantly ($P > 0.05$). The average sugar content in flowers of all Satsuma mandarin cultivars ranged from 4.13 mg to 4.96 mg. Sugar yield ranged from 240.8 kg/ha to 289.2 kg/ha and honey yield ranged from 301 kg/ha to 361.5 kg/ha. The present study shows that the studied cultivars of Satsuma mandarin are very attractive to honey bees due to the large number of flowers producing a large amount of nectar and that the Neretva Valley is a very good area for the production of this rare unifloral type of honey.

Keywords: *Citrus unshiu*, nectar secretion, honey yield, Croatia

SAŽETAK

U ovom istraživanju po prvi puta je praćena dinamika izlučivanja nektara unšijske mandarine na području Hrvatske. Istraživanje je provedeno od 2014. do 2016. godine na četiri sorte: Kawano Wase, Okitsu, Chahara i Zorica. Nektar je sakupljan uz pomoću mikrokapilara u razmacima od dva sata (8:00-18:00) tijekom 12 dana. Prosječne vrijednosti količine nektara kod sorti Okitsu (23,41 µL) i Kawano Wase (22,79 µL) nisu se statistički razlikovale ($P > 0,05$) od ostalih sorti, dok je značajna razlika ($P < 0,05$) utvrđena kod sorte Chahara (21,60 µL) u odnosu na Zoricu (20,01 µL). Količina izlučenog nektara kretala se od minimalno 5,00 µL do maksimalno 75,00 µL po cvijetu među istraživanim sortama. Uspoređujući koncentraciju šećera po sortama, najniža koncentracija utvrđena je kod sorte Zorica (18,43%) i značajno se razlikovala ($P < 0,05$) od ostalih, dok se srednje vrijednosti ispitivanog parametra kod sorti Okitsu (20,35%), Kawano Wase (20,66%) i Chahara (20,17%) nisu značajno razlikovale ($P > 0,05$). Prosječan sadržaj šećera u cvijetu svih sorti unšijske mandarine kretao se od 4,13 mg do 4,96 mg. Prinos šećera kretao se od 240,8 kg/ha do 289,2 kg/ha, a prinos meda od 301 kg/ha do 361,5 kg/ha. Ovo istraživanje pokazuje da su istraživane sorte unšijske mandarine vrlo atraktivne medonosnim pčelama zbog velikog broja cvjetova koji proizvode veliku količinu nektara te da dolina Neretve pruža mogućnost proizvodnje značajnih količina ove raritetne vrste meda.

Ključne riječi: *Citurs unshiu*, nektar, medonosni potencijal, Hrvatska

INTRODUCTION

Satsuma mandarin (*Citrus unshiu* Marc.) belongs to Sinocitrus (*Citrus reticulata* Blanco) (Xing-Tao et al., 2017) and represents one of the most important cultivar groups, of which there are more than 200 (Popović and Vego, 2010; Fujii et al., 2016). This species is divided into five main types: King mandarin, Mediterranean mandarin, Small-fruited mandarin, Satsuma mandarin, and Common mandarin. Various species, hybrids, and cultivars of Citrus spp. are widely grown in Mediterranean countries because they are very attractive to honey bees. Citrus is grown worldwide between 20° and 40° north and south latitudes. In Croatia, the main citrus growing area is between 42° and 44° north latitudes (Gugić and Cukrov, 2011), which is due to the influence of a warm sea current moving from south to north along the eastern Adriatic coast, and therefore this is one of the northernmost citrus growing areas in the world (Bakarić, 1983). On an area of 2500 hectares grow 2.5 million *Citrus* trees, 90% of which are different varieties of Satsuma mandarins, which are the main nectar flow of honey bees in springtime.

In the Neretva Valley, the flowering period of Satsumas lasts from late April to mid-May. Satsuma trees produce an abundance of flowers, more than 70 000 per tree (Prđun, 2017). Citrus pollination requirements vary widely (Sanford, 2003), ranging from self-pollination (Valencia oranges) to almost complete self-sterility (mandarin hybrid complex). Honey bees collect both, pollen (if present) and nectar from Citrus species. The flower is designed so that if the bee has previously visited a pollen-producing flower, some pollen is likely to be transferred to the next stigma visited (Sanford, 2003). The number of flowers per tree and the amount of nectar per flower vary greatly among Citrus cultivars (Dag, 2001). However, all Citrus species are among the most nectariferous commercially grown plants and produce a large amount of nectar (Free, 1993; Albrigo et al., 2012), with some orange cultivars producing as much as 73 µL per flower (Dag et al., 2001). Like most nectariferous plants, nectar secretion is significantly influenced by internal and external factors such as flower size and shape, nectar position, climate, soil type, and weather conditions. Air temperature, as the most important meteorological factor, has a significant influence on nectar secretion, and its optimum depends on the plant species and varies from species to species. A moderate increase in average air temperature can often have a positive effect on nectar secretion (Nocentini et al., 2013), while a greater increase imposes stress on plants that translate directly into a lower amount of nectar secreted (Scaven and Rafferty, 2013). Under such conditions, nectar secretion is reduced or ceases altogether. On the other hand, sugar concentration in flowers is not as dependent on external weather conditions and is more constant throughout the day and flowering period. However, according to Hoover et al. (2012), the amount of sugar per flower depends on the amount of nectar rather than sugar concentration, which can be strongly influenced by air temperature. In addition to meteorological conditions, the process of nectar secretion may also be influenced by other factors such as flower age and size, orchard density, time of day, and number of pollinators.

The flowering of Satsuma mandarin is closely related to weather conditions throughout the growing season, both in the current and previous year. In fact, weather conditions largely depend on the physiological state of the plant when it comes to flowering, which is ultimately related to nectar secretion. The number of nectar-gathering honey bees depends on many factors, the most important of which are weather conditions, distance to the nectar source, and colony strength. According to Ye and Zhong (1981), Citrus flowers begin to secrete nectar 3 to 5 days before full opening. Nectar production and its characteristics can vary significantly in response to sometimes minor changes in the environment, such as wind, temperature, soil moisture, or even the position of the flower on the plant (González-Porto et al., 2016). Based on the available scientific literature, this study represents the first record of nectar secretion dynamics and honey production potential of Satsuma mandarin cultivars from Croatia.

MATERIALS AND METHODS

Study area

The study was conducted in the Opuzen area (43°01'11.10" N; 17°31'14.56" E), Neretva Valley, Croatia, in April/May from 2014 to 2016 at the time of full bloom of Satsuma mandarin. According to the Köppen climate classification, the study area belongs to the Csa type, which means that this area has a Mediterranean climate with dry, hot summers, rainy and mild winters, and a regular change of seasons. The average annual temperature is 15.8 °C and the rainfall is 1300 mm with an average relative humidity of 69% (MHSC, 2016).

Nectar sampling

The amount of nectar secreted (µL) and sugar concentration (%) were recorded on 10 fully opened flowers of each cultivar in one measurement (Figure 2a). Six measurements were taken daily at two-hour intervals (8:00 to 18:00) over 12 days (3 days in 2014; 3 days in 2015; and 6 days in 2016). In total, we analyzed 2880 flowers of four Satsuma mandarin cultivars; Kawano Wase, Okitsu, Chahara and Zorica. Nectar was collected using the microcapillary method (Corbet, 2003) with calibrated 75 µL capillary tubes (Figure 2b). At least 24 h before nectar collection, flowers were isolated with a piece of fine mesh (approximately 100 x 80 cm; mesh size 1 mm) to prevent access by honey bees and other insects. Sugar concentration of each flower was measured directly in the field using a Mettler Toledo digital handheld refractometer, model Refracto 30 PX (Columbus, OH, USA). Nectar volume and sugar concentration were used to calculate the total sugar mass (mg) of each flower.

Weather data

Weather data such as air temperature, relative humidity and wind velocity were obtained from the Meteorological and Hydrological Service of Croatia (MHSC).

Honey production potential

The mass of sugar in the secreted nectar/flower was calculated from the volume and concentration of the solution. Concentration (mass/total mass, g sugar/100 g solution) was converted to mass/volume using a conversion table (Weasts, 1986). The number of Satsuma mandarin plants/ha (833 trees) was based on a plant spacing of 4 x 3 meters, with an average number of 70 000 flowers/tree (Prđun, 2017). The data were used to estimate honey production yield (kg/ha) of Satsuma mandarins in the Neretva Valley.

Statistical analyses

For all measured parameters, the mean, standard deviation, minimum and maximum, were calculated according to the procedure MEANS. The normality of the distribution of the studied parameters was tested using the Shapiro-Wilk test with the procedure UNIVARIATE. A generalized linear model (GLM) was employed to analyze the dependent variables, namely nectar volume and sugar concentration. The effects of two fixed factors, cultivar (n = 4) and year (n = 3), were examined. Post hoc comparisons using Bonferroni tests were then conducted to identify significant differences. Statistical data were processed using the SAS 9.4 program (SAS, 2012).

RESULTS

Weather conditions

The average air temperature during the study in 2014 was 15.7 °C (min. 14.7 °C; max. 16.9 °C) with an average humidity of 81% (range of 76% to 85%), which resulted in very good nectar secretion of all studied cultivars (Figure 1). However, in 2015, Satsuma mandarin flowered in the first half of May, and at that time an average daily air temperature of 19.4 °C (min. 18.7 °C; max. 20.2 °C) and relative humidity of only 53% (range 41% to 61%) were recorded, resulting in significantly lower nectar secretion compared to 2014. That year also recorded daily winds from the west and south (1.7-3.1 m/s), which ultimately had a significant impact on the duration of flowering, but also on low nectar secretion. In 2016, the average air temperature at flowering was 14.7 °C (min. 11.7 °C; max. 16.0 °C) with an average humidity of 65% (with a range of 47% to 78%), and this year nectar secretion was higher but not as abundant as in 2014.

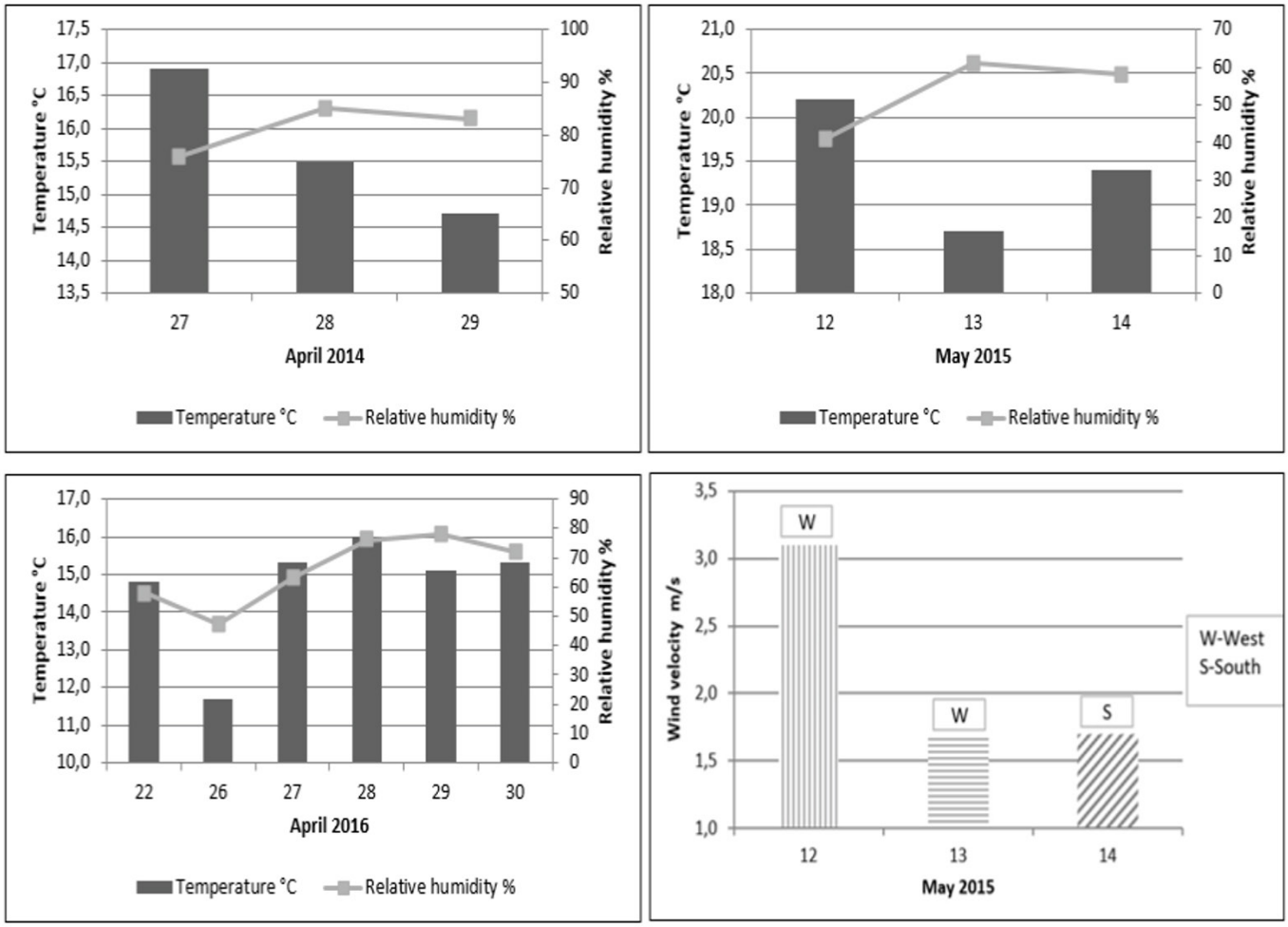

Figure 1. Average air temperature (°C), relative air humidity (%), wind velocity (m/s) and direction recorded during the study

Nectar secretion and volume

When comparing the amount of secreted nectar across all studied cultivars per year, the highest mean value was observed in 2014, which was significantly different ($P < 0.05$) from the values determined in 2015 and 2016. However, there were no significant differences between 2015 and 2016. This parameter exhibited a range from a minimum of 5.00 µL to a maximum of 75.00 µL, with the lowest measurement recorded in 2016 (Table 1).

A significant difference ($P < 0.05$) was found when comparing the amount of nectar secreted by different cultivars. Specifically, Okitsu and Kawano Wase cultivars showed a significant difference, while their nectar quantities did not significantly differ from the other cultivars. Additionally, another significant difference was observed between the Chahara and Zorica cultivars (Table 2), with the Chahara cultivar exhibiting a higher mean nectar quantity compared to the Zorica cultivar.

Table 1. The amount of secreted nectar (µL) of all cultivars during 2014-2016

Year	N	x̄	Sd	Minimum	Maximum
2014	720	24.48[a]	8.99	5.00	46.00
2015	720	21.27[b]	6.05	5.00	44.00
2016	1 440	21.03[b]	8.28	5.00	75.00

The different letters in columns showed significant differences among means at $P < 0.05$

Table 2. The amount of secreted nectar (µL) per cultivar during 2014-2016

Cultivar	N	x̄	Sd	Minimum	Maximum
Okitsu	720	23.41[a]	7.93	7.00	75.00
Kawano Wase	720	22.79[a]	8.10	5,00	56.00
Chahara	720	21.60[b]	7.83	6,00	50.00
Zorica	720	20.01[c]	8.17	5,00	46.00

The different letters in columns showed significant differences among means at $P < 0.05$

As shown in Table 3, the mean values of sugar concentration for all cultivars showed that they were significantly different ($P < 0.05$). The highest value was found in 2016 (21.62%) and was significantly different from 2014 (18.29%) and 2015 (18.07%), between which there were no significant differences.

When sugar concentration was compared by cultivar (Table 4), the lowest concentration was found in the Zorica cultivar (18.43%) and differed significantly ($P < 0.05$) from the others, while the mean values of the studied parameter for Okitsu (20.35%), Kawano Wase (20.66%) and Chahara (20.17%) cultivars were not significantly different from each other.

Honey production potential

Based on the data obtained, the average sugar content of the flowers of all Satsuma mandarin cultivars ranged from 4.13 mg to 4.96 mg (Table 5). Sugar yield calculated in this study ranged from 240.8 kg/ha to 289.2 kg/ha and honey yield ranged from 301 kg/ha to 361.5 kg/ha.

Table 3. Sugar concentration (%) in all cultivars during 2014-2016

Year	N	x̄	Sd	Minimum	Maximum
2014	720	18.29[a]	4.41	2.50	37.20
2015	720	18.07[a]	3.34	6.00	34.30
2016	1 440	21.62[b]	6.34	4.70	56.80

The different letters in columns showed significant differences among means at $P < 0.05$

Table 4. Sugar concentration (%) per cultivar during 2014-2016

Cultivar	N	x̄	Sd	Minimum	Maximum
Okitsu	720	20.35[a]	5.83	5.50	43.30
Kawano Wase	720	20.66[a]	6.19	5.40	56.80
Chahara	720	20.17[a]	5.52	4.70	48.40
Zorica	720	18.43[b]	4.13	2.50	34.80

The different letters in columns showed significant differences among means at $P < 0.05$

Table 5. The amount of sugar (mg) per flower of all cultivars

Year	N	x̄	Minimum	Maximum
2014	720	4.88	0.37	13.50
2015	720	4.13	0.57	10.13
2016	1 440	4.96	0.80	16.48

The different letters in columns showed significant differences among means at $P < 0.05$

Figure 2a. Nectar secretion on the disk of Satsuma mandarin flower; **Figure 2b.** Nectar collection by microcapillary method

DISCUSSION

In favourable years, Satsuma mandarins, like other Citrus cultivars, secrete large amounts of nectar per flower (Free, 1993; Albrigo et al., 2012; Prđun, 2017), up to 73 µl in some orange cultivars (Dag et. al., 2001), so they are considered one of the most nectariferous plant species. This was also confirmed in this study, where the maximum secreted amount of nectar per flower was 75 µL. However, as in most nectariferous plant species, nectar secretion is significantly affected by weather conditions. Air temperature, as the most important meteorological factor, has a significant influence on nectar secretion, and its optimum depends on the plant species and varies among species (Petanidou and Smets, 1996; Pacini et. al., 2003). A moderate increase in average air temperature can often have a positive effect on nectar secretion (Nocentini et al., 2013), while at higher temperatures plants are subjected to some stress that is directly reflected in a lower amount of nectar secreted (Scaven and Rafferty, 2013), with the exception of some nectariferous plants from desert areas (Nuru et al., 2015). Under such weather conditions, secretion decreases (Keasar et. al., 2008) or stops altogether in most plant species (Petanidou and Smets, 1996), as shown in the present study in 2015. Another unfavorable factor that year was the wind from southern and southwestern directions. Wind was recorded on all study days and had a largely negative effect on nectar secretion. Especially the south and west winds had a negative effect on nectar collection, as the nectar dried faster due to the specific flower structure. In addition, frequent winds made the flight of an unfortunate collector impossible, which ultimately led to reduced gathering activity of honey bees. On the other hand, Southwick (1983), Villarreal and Freeman (1990), and Nocentini et al. (2013) note that sugar concentration in the flower does not depend so much on external conditions and is more uniform during the day and the flowering period. However, according to Hoover et al. (2012), the amount of sugar per flower depends on nectar quantity rather than nectar concentration, although air temperature can have a significant influence. As in other plant species, flowering of Satsuma mandarin is closely related to weather conditions throughout the growing season, e.g., in the current year and the previous year. In fact, weather conditions largely depend on the physiological state of the plant when it comes to flowering, which is ultimately related to nectar secretion. Air temperature and relative humidity, and especially wind, can have a direct negative effect on honey bees foraging, but also on the condition of the plant itself and thus on the availability of nectar (Winston, 1987).

The optimal conditions for nectar secretion of most plant species are at a temperature of 20 °C to 25 °C and a relative humidity of 60% to 80% (Šimić, 1980). However, this is not the case for Satsuma mandarin, as it flowers most frequently at the end of April, when the average daily temperature in the Neretva Valley does not exceed 20 °C, which is confirmed by the data obtained in this study (Figure 1). It was also found that negative wind effects have a significant impact on nectar secretion of Satsuma mandarin due to the particular structure of the flower. The largest amount of nectar was formed in 2014, when the air temperature was between 14.7 °C and 16.9 °C and the average relative humidity was above 80%. In addition to meteorological conditions for nectar secretion, other factors such as flower age (Torres and Galetto, 1998; Valtuena, et. al., 2007), flower size (Longo and Fischer, 2006), plant density (Klinkhamer, 2004), flower sex (Liu et al., 2002; Carlson, 2007), time of day (Macukanovic-Jocic, et.al, 2004; Valtuena et al., 2007), and number of pollinators (Torres and Galetto, 1998; Valtuena, et al., 2007; Keasar et. al., 2008).

According to Crane (1990), only 16% of all plant species provide food for bees, and not all are equally important for bees and honey production. Only 1.6% of nectariferous plant species are the source of most of the world's known honey. This means that there are only a few important nectariferous plant species in each geographic area and it is of great importance to determine their honey potential (Nuru et al., 2017). The most important factors affecting the attractiveness of the flower to pollinators are the number of flowers and the sugar concentration in the nectar. When the sugar concentration is less than 20%, honey bees require much more energy to extract excess water from the nectar and convert it into honey (Free, 1993). The sugar concentration values obtained in this study are in agreement with those of Free (1993), according to which the average sugar concentration in bitter oranges (*C. aurantium*) is 15%, in lemons (*C. lemon*) 15-18%, in grapefruits (*C. paradisi*) 16%, in mandarins (*C. reticulata*) 22%, and in sweet oranges (*C. sinensis*) 11-18%. The main reason for the lower average amount of secreted nectar and, consequently, the lower sugar concentration in 2016 in the Zorica variety could be related to the large number of smaller flower buds per tree compared to other studied varieties.

Comparing the analysed nectar quantity and sugar concentration with other plant species, it can be seen that Satsuma mandarin is very rich in nectar with a yield of 250 to 500 kg/ha and belongs to category V according to Ricciardelli D'Albore and Intoppi (2000). Farkasz and Zajacs (2007) reported that black locust (*Robinia pesudoacacia*) secretes on average 1.5 ml of nectar with 2 mg of sugar and yields 1000 kg/ha, while sweet chestnut (*Castanea sativa*) has 1 ml of nectar with 22% sugar and a honey yield of 100 to 250 kg/ha. According to Crane et al. (1984), lime (*Tilia* spp.) yields 90-1200 kg/ha honey, depending on the species. The most nectariferous field crops are phacelia (*Phacelia tanacetifolia*) with a yield of 500-1000 kg/ha (Petanidou, 2003) and sweet clover (*Melilotus albus*) with 250-500 kg/ha (Király, 1994). Plant species with a smaller number of flowers per tree consume less energy and nutrients at the time of flowering and consequently release a greater amount of nectar (Karp et al., 2004). Some plant species also have the ability to reabsorb nectar, thereby conserving energy (Burquez and Corbet, 1991, Nepi and Stypiczynska, 2008), while in some cases nectar secretion ceases when there are insufficient pollinators (Edge, 2010). The flower of Satsuma mandarin is sterile and does not need pollinators to bear fruit, but it secretes large amounts of nectar, while in most other plant species secretion continues until the flower leaves (Manetas and Petropoulou, 2000). Nectar secretion of the Satsuma mandarin occurs only once, i.e., after the nectar is taken up by the honey bee or another pollinator, the plant stops secreting nectar. Since there are no data on cultivated areas by varieties in the Neretva Valley, according to estimates of the 1700 ha, between 511.7 and 614.5 tons of Satsuma mandarin honey could be produced during the study period.

CONCLUSION

It can be concluded that the Neretva Valley is a very good area for the production of this rare unifloral honey type. The present study shows that the studied cultivars of Satsuma mandarin are very attractive for honey bees due to the following characteristics: large number of flowers produced by the plants, large flowers and relatively large nectaries, which provide large amounts of nectar. Therefore, local beekeepers should make even better use of the possibility of producing Satsuma mandarin honey, since mainly mixed *Citrus* honey is sold on the world market.

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