Botanical Origin and Quantity of Pollen Load on Satsuma Mandarin (*Citrus unshiu* Marc.) Honey Bee Forage

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Summary

Although Satsuma mandarin secretes large amounts of nectar and is considered as excellent honey bee plant species, its anthers are either sterile or produce very small amount of pollen. Therefore, honey bees collect pollen from other plant resources as well. The aim of this study was to determine the botanical origin of pollen loads collected with pollen trap during Satsuma mandarin (*Citrus unshiu* Marc.) honey bee forage, and to investigate relating foraging parameter (pollen collection time). The study was carried out on Carniolan honey bee (*Apis mellifera carnica* Pollmann, 1879) colony during April in 2014 and May 2015 in Neretva Valley, Croatia. Pollen of 12 plant species was identified in analysed samples. Results have revealed that a total pollen mass was significantly higher ($p<0.05$) in the morning hours compared to the sampling carried out in the afternoon. The largest content of the pollen load in the morning originated from *Crepis biennis* L. and *Citrus sinensis* L., while the pollen grains of *Cerastium glomeratum* Thuill. and *C. sinensis* were the most dominant pollen components of investigated pollen loads in the afternoon. Although Neretva Valley has almost monocultural Satsuma mandarin orchards, a notable amount of pollen originating from other plant species and non-sterile *Citrus* were observed in analysed pollen loads

Key words

Satsuma mandarin forage, *Apis mellifera carnica*, pollen load, botanical origin

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Introduction

Pollen, as a small portable plant organ produced in anthers of a flower, plays an important role in the honey bee colony. Honey bees use pollen as food for all developmental stages in the hive (Morais et al., 2011), because it provides an optimal ratio of all nutrients (Schmidt et al., 1987; Garcia-Garcia et al., 2004). In the bee hive, pollen is being converted into proteinaceous brood food (bee-bread) by nurse bees (Crailsheim et al., 1992), which is crucial for the brood developement in a honey bee colony. The need for pollen within a colony is closely correlated with the relative amounts of larval brood and stored pollen present in the hive (Dreller and Tarpy, 2000). Honey bees also need pollen during the working period when the hypopharyngeal gland and wax glands are in intense function. If the pollen source is insufficient, the brood development is poor and consequently, the number of honey bees is declining. In that case, the bees first consume deposited stocks of the bee bread around brood, and afterwards they spend the food reserves from their body which may subsequently result of larvae cannibalism in the times of pollen shortages (Schmickl and Crailsheim, 2001). In some plants species, extreme amounts of pollen components can be found, so the protein content may vary from 2-61% (Nicolson, 2011), sugar 15-50%, and starch up to 18%. The fat content in the pollen also depends on the plant source and ranges from 0.8 to 18.9% (Roulston and Cane, 2000). Other minor components are minerals and trace elements, vitamins and carotenoids, phenolic compounds, flavonoids, sterols and terpenes (Feas et al., 2012). For this reason pollen collected from different plant species has different nutrient value and can vary due to their botanical and geographic origin (Almaraz-Abarca et al., 2004).

Honey bees collect most of the pollen during the spring when the majority of the plant species are blooming and when the weather conditions are favorable for colony development and growth. The pollen foragers make between 10 to 15 flights per day to collect pollen, which significantly depend on the weather conditions and the strength of the colony. When honey bee foragers find the source of the pollen, they use signaling movements in the hive (Toufailia et al., 2013) in order to encourage other pollen foragers to fly out and to collect pollen (Gardner et al., 2008; Donaldson-Matasci et al., 2013; Edrich, 2015). The mass of the pollen load that honey bee can handle ranges from 10 to 30 mg (Winston, 1987; Pernal, 2000). The pollen composition of pollen loads depends on the climate, soil type and honey bee activity (Feás et al., 2012), as well as the weather conditions, time of the day when pollen is collected, season, type of pollen trap, and plant species.

Determination of botanical and geographical origin represents the basis for understanding the quality of pollen. Beekeepers are usually aware of the nectar sources in a particular foraging area, but often less is known about the pollen sources (Dimou et al., 2006). Although Satsuma mandarin secretes large amounts of nectar and is considered as an excellent nectariferous plant, its anthers are either sterile or have a very small amount of pollen grains (Bakarić, 1983; Prđun, 2017). Consequently, Satsuma mandarin flowers cannot provide enough pollen for development of honey bee colonies during its blooming period.

Given the general lack of scientific reports on *Citrus* honey bee forage, the aim of this study was to determine the botanical origin of pollen loads collected with pollen trap during Satsuma mandarin (*C. unshiu* Marc.) honey bee forage, and to investigate relating foraging parameter (pollen collection time). According to the data from available scientific literature, this study represents the first record of the botanical origin of pollen loads collected during Satsuma mandarin (*C. unshiu*) honey bee forage.

Materials and methods

Study area

The study was carried out in the area of Satsuma mandarin orchards located in the Neretva Valley, Opuzen (43°01'11.10" N; 17°31'14.56" E), during the April 2014 and May 2015.

Pollen load sampling

A metal pollen trap was fixed on the 10 frame Langstroth-Rooth hive entrance of the Carniolan honey bee (*Apis mellifera carnica* Pollmann, 1879) colony in the experiment during the Satsuma mandarin bloom period. The pollen load samples were removed from the legs of the honey bees on a rack fitted in trap; as bees passed through the trap the loads on their legs fell down. The pollen load was collected twice a day, in the morning (8:00 a.m. to 1:00 p.m.) and afternoon (1:00 p.m. to 6:00 p.m.) during six days in 2014 and three consecutive days in 2015. After collection, the pollen loads were stored in plastic vials and stored at -18°C until further laboratory analysis.

Pollen load mass

In order to determine the mass of collected pollen loads, they were weighed individually using the Kern ALJ 220-4NM digital scale (range 0.1 mg - 220 g). After classification of pollen loads by their colors using a small drawing brush, each group was separately weighed and its proportion in the total pollen sample size was recorded. The slides for melisopalynological (pollen) analysis were prepared according to the guidelines of the International Commission of Bee Botany (Louveaux et al., 1978). One grain of pollen from each colored group was extracted and placed on the microscopic slide previously labeled with the date and color marking. Then the pollen grain was caught by one to two drops of distilled water and mixed until the entire pollen load had completely dissolved.

Pollen analysis

The pollen analysis was performed using a light microscope Hund h 500 (Wetzlar, Germany) at a magnification of 400-1000 x. Dino-Lite digital camera and Dino Capture 2.0 software were used to measure the pollen grain size. The pollen grains were identified according to the literature data (Ricciardelli D'Albore, 1998; Von der Ohe and Von der Ohe, 2003) and internal pollen grain reference library (University of Zagreb Faculty of Agriculture).

Statistical analysis

Descriptive statistical analysis was performed to calculate the mean, minimum and maximum values and the standard deviation for the pollen load mass using SAS 9.4 software package.

Results and discussion

The results on the pollen load collected with pollen trap (Table 1) have revealed that the average total mass in 2014 was higher in the morning (30.92 g) and significantly different ($p<0.05$) compared to the sampling carried out in the afternoon (8.78 g). These results are in agreement with findings reported by Grabowski et al (2000) and Hoopingarner and Waller (2003), whose studies indicate that pollen of many plant species can only be collected at higher relative air humidity, which is mostly in the morning hours. This was also confirmed by Valentić (2009) who reported the largest amounts of pollen loads on *Salvia officinalis* honey bee forage in the morning

Table 1. Total mass of pollen loads (g) collected in the morning and afternoon during April 2014 and May 2015

Year	Time	$\overline{x}$	Sd	Range
2014	morning	30.92^{a}	18.09	7.13 - 51.17
	afternoon	8.78^{b}	3.31	2.99 - 11.90
2015	morning	10.55^{a}	3.71	6.28 - 12.97
	afternoon	5.18^{a}	4.77	0.48 - 11.24

* The mean values of pollen load mass were upgraded within and were not compared between the investigated years. The different letters in columns showed significant differences among means at p≤ 0.05

hours. Mahmood et al. (2017) investigated *Brassica campestris* honey flow and found that the best period for pollen collection is 10:00-12:00 a.m. Honey bees mix the freshly collected pollen with nectar and secretions from the salivary gland before storing it in the basket on their legs and making it sticky (Bogdanov, 2009). This finding have been evidenced by Garcia-Garcia et al. (2004) suggesting that bees can collect pollen from anemophilic plant species like *Populus alba* and *Quercus suber*, that produce very dry and non – cohesive pollen.

In the following year (2015), the average weight of the investigated parameter in the morning (10.55 g) did not differ significantly (p>0.05) from the weight of the pollen load (5.18 g) collected in the afternoon. This could be associated with a strong wind of southern direction that was blowing all days during Satsuma mandarin flowering (Meteorological and hydrological service of Croatia, 2016). This also directly affected the significantly smaller mass of pollen load that bees were collected, especially in the morning hours. According to the study by Prđun (2017), more than 70 plant species bloom at the time of Satsuma mandarin honey flow in Neretva Valley. However, the number of plants intensely utilised by honey bees were rather small (12). This can be related to the amount of nutrients in certain types of pollen, suggesting that some are of better quality for honey bees than others (Pasquale et al., 2013). This fact is supported by Roulston and Cane (2000) who showed that honey bees collect pollen from plant species with higher nutritional content.

Based on the pollen analysis of the collected loads during 2014 and 2015, the pollen from 12 plant species was found. Seven species were determined in the morning loads (Table 2) and 11 species during the afternoon (Table 3). In 2014 collected pollen loads during morning hours in first four days originated exclusively from

Table 2. The type and content (%) of pollen determined in the pollen load samples (n=9) collected in the morning hours during 2014 and 2015

Date	Pollen types (%)						
	Cerastium glomeratum	*Citrus paradisi*	*Citrus sinensis*	*Crepis biennis*	Fabaceae	*Fraxinus ornus*	*Quercus ilex*
23.4.2014				100.00			
24.4.2014				100.00			
25.4.2014				100.00			
27.4.2014				100.00			
28.4.2014				100.00			
30.4.2014	13.94			4.40	0.21	72.34	9.10
Total content (%)	2.32			84.07	0.04	12.06	1.52
13.5.2015			100.00				
14.5.2015			100.00				
15.5.2015		13.51	81.19	0.38			4.92
Total content (%)		4.5	93.73	0.13			1.64

Table 3. The type and content (%) of pollen determined in the pollen load samples (n=9) collected in the afternoon hours during 2014 and 2015

Date	Pollen types (%)										
	Capsela bursa pastoris	*Cerastium glomeratum*	*Cistus incanus*	*Citrus paradisi*	*Citrus sinensis*	*Crepis biennis*	*Dactylis glomerata*	*Fraxinus ornus*	*Marubium* spp.	*Quercus ilex*	*Rhamnus* spp.
23.4.2014	1.32	15.32				83.37					
25.4.2014		73.99				1.07	5.60	19.35			
27.4.2014		48.05				3.42		48.52			
28.4.2014		0.55				4.06	1.92	31.29	30.18		32.01
29.4.2014						51.29				48.71	
30.4.2014		15.24	53.03			1.38	2.31	28.04			
Total content (%)	0.22	25.53	8.84			24.1	1.64	21.2	5.03	8.12	5.34
13.5.2015				100.00							
14.5.2015					100.00						
15.5.2015					99.62		0.38				
Total content (%)				25.00	74.91		0.09				

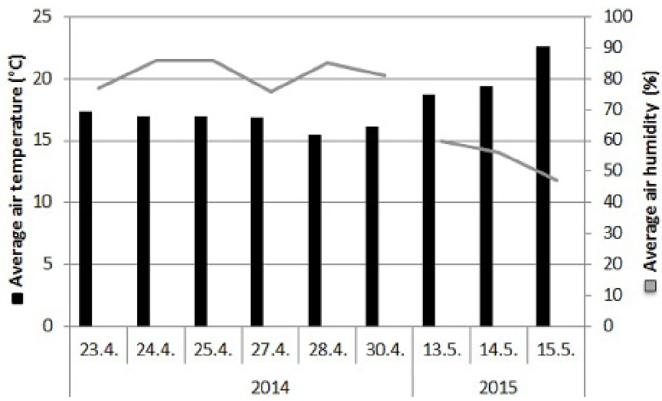

Figure 1. The average air temperature and humidity during collection of pollen load samples

C. biennis (100.00%), while the last day of sampling, with the exception of *C. biennis* (4.40%), showed the largest content of the pollen loads originating from *Fraxinus ornus* L. (72.34%), *Cerastium glomeratum* (13.94%), and *Quercus ilex* L. (9.10%). It was also observed that during the experimental period in the year 2014 *Citrus* spp. pollen was not present in the collected samples.

In the following year, the *C. sinensis* pollen was 100.00% during two first days, while third day it was 81.19% of C. sinensis pollen and the rest was pollen of the same species as in previous year exept one species. In 2014, there was predominantly a *C. biennis* pollen, while that was not the case in 2015 when this pollen type was registered during the last sampling day with a content of only 0.38%.

The results from 2014 suggest that the main reason for such high content of *C. biennis* was lower average air temperature and higher relative air humidity during Satsuma mandarin bee forage when compared with weather conditions in 2015 (Figure 1). More specifically, these weather conditions favored the *C. biennis* nectar secretion and pollen production. Contrary to the above, during the days with high air temperature *C. biennis* closes its capitulas flowers early in the morning hours and becomes inaccessible as a pollen source for honey bees (Prđun, 2017).

As presented in Table 3, five additional plant species were found in the afternoon sampling period in comparison to sampling in the morning hours: *Capsella bursa pastoris* (L.) Medik., *Cistus* x *incanus* L._*Dactylis glomerata* L., *Marubium* spp. and *Rhamnus* spp., along with the pollen type from the Fabaceae family. The results have revealed that the most of the pollen loads in 2014 were collected from *C. glomeratum* (25.53%) and *C. biennis* (24.01%) while in the following year of the study the pollen from non - sterile *Citrus* species was dominating, such as C. *sinensis* (74.91%) and *C. paradisi* (25.00%). Although Neretva Valley has almost monocultural Satsuma mandarin orchards on 2.500 hectars with over 2.5 million trees, notable amounts of pollen originating from other plant species and non-sterile *Citrus* were observed in analysed pollen loads. Sufficient quantities of pollen are very important for the further development of the colonies and the next honey forages. This type of research can also provide more data of plant species present in other types of uniflorous honey, especially obtained from the plant species with sterile anthers.

Conclusions

In this study we have demonstrated that the pollen load collection by honey bees comes from more places in the morning hours because of the higher relative humidity when the pollen is stickier. During blooming of Satsuma mandarin the pollen of different plant species appear, which directly depends on the weather conditions. In years with humid weather conditions dominant was pollen of one and two-year plant species, *C. biennis and C. bursa pastoris*, while in dry weather condition, which was recorded in second year of the study, pollen of woody plant species, primarily from *Citrus* spp. (orange and grapefruit) and *F. ornus* was dominant. Although Neretva Valley has almost monocultural Satsuma mandarin orchards that due to sterile anthers do not provide pollen for pollinators such as honey bees, pollen of other plant species and non-sterile *Citrus* spp. (orange) was quite present in the collected pollen load. This is very important for obtaining the better pollen analysis of this rare unifloral honey type.

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