Saudi Journal of Biological Sciences 24 (2017) 1470–1474

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Nectar secretion dynamics of *Ziziphus nummularia*: A melliferous species of dry land ecosystems

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ARTICLE INFO

Article history:
Received 19 December 2016
Revised 3 January 2017
Accepted 25 January 2017
Available online 3 February 2017

Keywords:
Ziziphus nummularia
Flower age
Nectar
Total soluble solids
Weather conditions
Hot-arid environment

ABSTRACT

Nectar is used as raw material for the production of honey and as significant reward in the relationship between bees and plants during pollination. Therefore, it is important to investigate its abundance, dynamics and associated governing factors. Weather conditions are known to influence nectar production, and predicted climate changes may be responsible for future declining in total yield from beekeeping activities. We investigated nectar production as total soluble solids (TSS) of well-known species for honey production, *Ziziphus nummularia* in a hot-arid environment of Saudi Arabia. Data on nectar samples from bagged flowers of different stages during two blooming seasons, 2013 and 2015 were collected on weekly bases, and the data were correlated with weather conditions (temperature, relative humidity, and wind). A significant difference in TSS amount has been obtained, with 1-day old flowers displaying the higher content. TSS production was varied along the different day intervals, for both years, with a peak of production in the afternoon. In our results, nectar production was not correlated to temperature and wind, but was significantly negatively correlated with relative humidity. According to the current and future weather forecasting conditions, understanding of the relationship between weather conditions and nectar availability turned out to be important predictive information that may be interpreted into an economic projection of incomes from beekeeping activities.

1. Introduction

The importance of nectar is usually neglected as an important resource when dealing with agricultural practices. Generally, there is a big information gaps regarding availability of nectar, frequency and time of production, characteristics of nectar secreting flowers, and its correlation with weather conditions. Nectar is often crucial for pollination of economically important crops (Farkas and Orosz-Kovács, 2003) and for the production of honey, a commodity, which has a double value for national commerce and international trade (Jones, 2004). Nectar is a widespread resource usually secreted by dedicated structures, nectaries, present in most of the angiospermic flowers. These plants depend on animals (Zoophilous angiosperms) for pollination service. Zoophilous angiosperms account for about 86% of the total angiosperms (Hu et al., 2008). Nectar plays an important role in plant-animal interaction resulting in plants attracting pollinators (Heil, 2011). Bees could be attracted to flowers from a very long distance and select best sources of nectar to produce large quantities of honey (Grüter and Farina, 2007).

Nectar is sugar rich solution containing amino acids and some secondary metabolites such as alkaloids and phenolic compounds. Chemical composition of nectar has been reviewed elaborately (Nicolson et al., 2013). Even though new classes of substances are frequently detected in nectar, renewed interest is paid to the presence and function of secondary compounds in nectar secretion (Escalante-Pèrez and Heil, 2012). Jasmonic acid and extracellular

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2017.01.059
1319-562X/

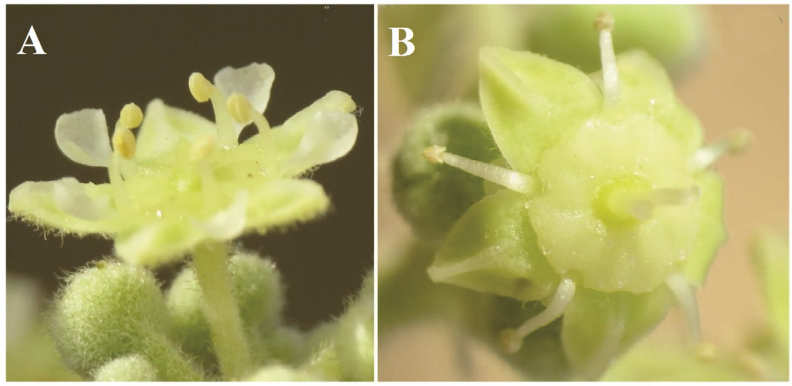

Fig. 1. Flowers of *Ziziphus nummularia* in central Saudi Arabia with different ages. On the left, flower that just started the anthesis, 1-day old (A): stamens are standing and anthers look fresh and full of pollen grains. On the right, a 2-days old flower (B): stamens curved downward and anthers changed colour after starting to dry.

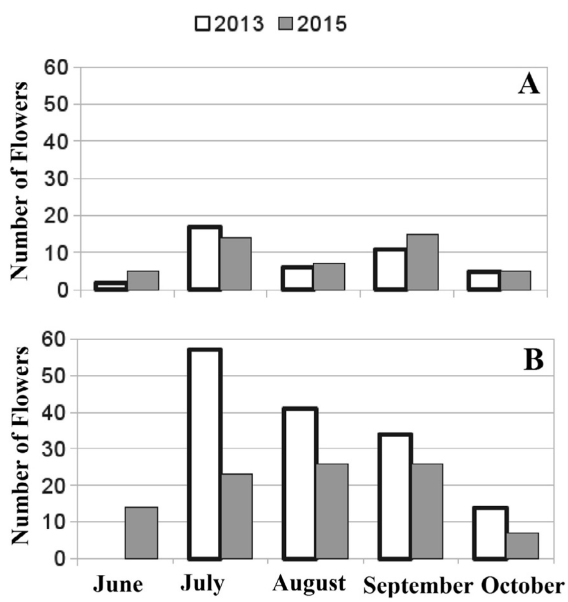

Fig. 2. Seasonal distribution of *Ziziphus nummularia* flowers in central Saudi Arabia, only samples without nectar, in 2013 (white bars) and 2015 (grey bars). The distribution of empty (A) flowers is represented in the upper graph, the distribution of empty (B) flowers in the lower graph.

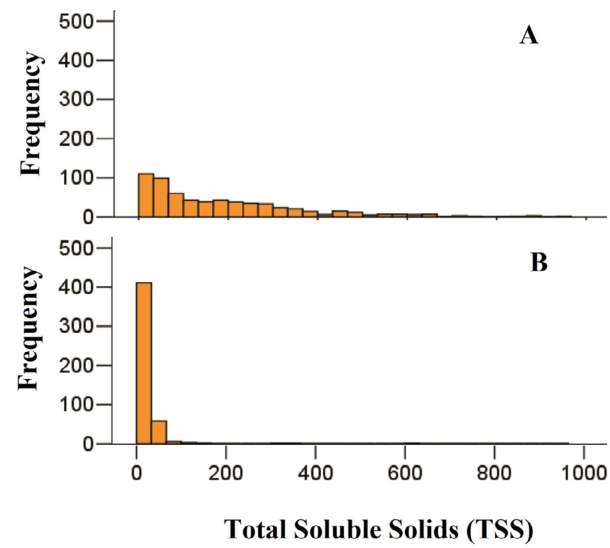

Fig. 3. Frequency of *Ziziphus nummularia* flowers with different TSS content. Upper graph refers to one day flowers; lower graph to two day flowers.

invertase were found to be involved in nectar production of *Brassica napus* and *Arabidopsis thaliana* flowers (Radhika et al., 2010 and Ruhlmann et al., 2010). Secretion of nectar may follow a diurnal rhythm correlated to the visits of pollinators (Boisvert et al., 2007; Giovanetti et al., 2015), and to weather conditions including temperature and relative humidity, both affect its volume (Langenberger and Davis, 2002; Pacini et al., 2003). Information related to flower age, weather conditions, and nectar secretion are so limited to help in the prediction trends for nectar secretion and in guiding beekeepers for appropriate measures and practices. Figuring out nectar productivity could lead to extrapolate honey production potentials (Adgaba et al., 2012; Alqarni et al., 2015; Nuru et al., 2015). Honey bees still able to survive and produce honey under adverse harsh climates just when and where melliferous floras are present (Awad et al., 2016). Moreover, the presence of wild melliferous flora may help the conservation of pollinators under such climates (Alqarni et al., 2017).

Ziziphus nummularia (Burm. f) is one of the frequent melliferous species in arid areas of central Saud Arabia and forms dense aggregations of shrubs. It starts flowering around June and continue till October. Beekeepers in Saudi Arabia move their honey bee colonies into desert oasis, where this species occurs densely to harvest unifloral *Z. nummularia* honey, or locally called sidr honey of high market demand (Mohammed et al., 2015). *Z. nummularia* produces numerous individual flowers that last for two days. Results of a previous work (Alqarni, 2015) reported a more conspicuous nectar production on the first day of anthesis with different trends along the day.

For nectar production, weather conditions are critically important particularly in arid environmental conditions. Plants adapted to arid environment, may potentially have higher resistance to changes in temperature and relative humidity that could lead to constant nectar production. The objective of this work was to analyse the availability of nectar in *Z. nummularia* flowers in relation to flower age and weather conditions, and to compare flowering periods of different years with variable weather conditions. Such study will assist in understanding the dynamics of nectar secretion, and providing data for other studies aiming to establish future scenarios for better beekeeping practices in arid environment.

2. Materials and methods

Data has been collected in an oasis, named Rawdhat-Khoraim (25°30′25″N, 47°46′30″E), located in the middle of a vast desert, 120 km north-east of Riyadh city, Saudi Arabia. It is known as "valley of flowers" with a high diversity of about 112 plant species (Al-Farraj et al., 1997), possibly increased in the past 20 years due to protection measurements. The species *Z. nummularia* belongs to family Rhamnaceae and is quite widespread in grazed areas of arid and semi-arid regions of Saudi Arabia. The economic value of this species lies on its medicinal properties and its use as melliferous species. The oasis is partly protected as a national park. Data were collected in the protected area where limited human activities permitted, such as beekeeping.

The research has been carried out during the two flowering seasons from June to October, in 2013 and 2015. At the site, five *Z. nummularia* shrubs were marked for subsequent nectar measurements, and the same marked individuals were used for the two years data recordings. Individual flowers were bagged before dawn with bridal veil (Wyatt et al., 1992). During the day, two flowers were selected on each plant: one newly opened flower (1-day old, 1D), and the second was already open (2-days old, 2D) (Fig. 1). These flowers were monitored throughout the day, undergoing 5 times nectar measurements (at sunset, forenoon, noon, afternoon, and sunrise). Data recording was done 15 times in 2013 and 14 times in 2015, and a total 1450 records on 50 flowers during the two years.

The nectar concentration as total soluble solids (TSS) was measured in each flower using the washing technique according to (Mallick, 2000). Seven μl of deionized water were carefully deposited on each flower using a calibrated micropipette. The added water was retrieved three times to dissolve the highly concentrated nectar. An automatically temperature-compensated digital handheld refractometer (Reichert®, model 13950000, USA) was used to measure the resulted solution concentration. After each measurement, distilled water was added for washing, the instrument reading was calibrated to 0.00, and a new tip was used for each flower. In each flower, the sugar mass in the secreted nectar was determined by the volume and concentration of the solution measured. When measuring their TSS, the 2D flowers were washed using deionized water at dawn. This procedure was followed to remove crystalized nectar from the previous day.

The concentration was converted to mg following the equation: TSS (mg/flower) = TSS% $\times$ 7/100 to get the nectar amount in TSS (μg/flower). Weather data such as: temperature, relative humidity (RH) and wind speed were obtained from the close King Khaled International Airport Weather Station, which is approximately 50 km away from the study area.

Statistical analyses were performed on untransformed data (transformation did not help at normalising data), applying non-parametric tests when heterogeneity of variances were significant. We used the software SPSS (version 13.0).

3. Results and discussion

We first analysed individual flowers, under their state of anthesis. According to Alqarni (2015), *Z. nummularia* flower age is two days. Therefore, we compared 1D and 2D flowers for presence of nectar and TSS quantity. The percentage of flowers without nectar was different between the two categories. In 1D flowers, the overall percentage of flowers without nectar was about 15%, while in 2D flowers the percentage was significantly higher (25%) (Pearson Chi-Square$_{(1,N = 1450)}$ = 94,456; $p < 0.0001$).

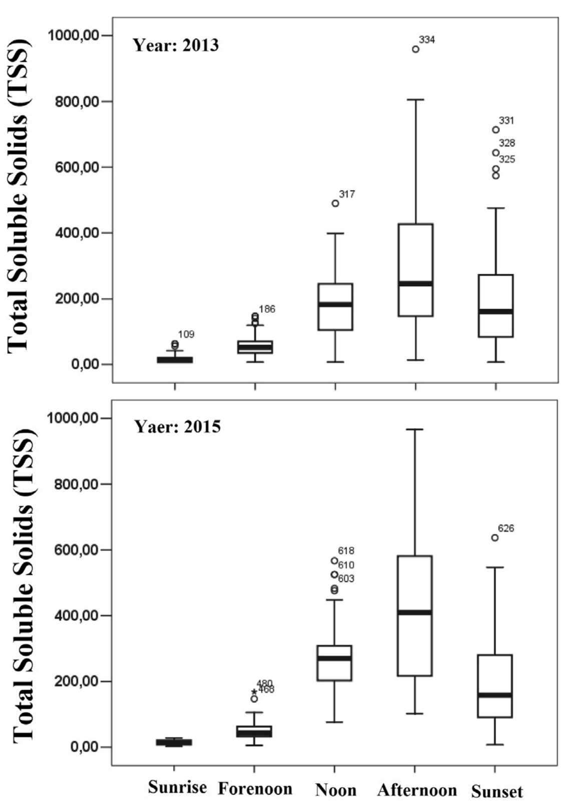

Fig. 4. Average of total soluble solids (TSS) quantities in *Ziziphus nummularia* one day flowers during the day in the two seasons in central Saudi Arabia: upper graph represents data collected in year 2013, lower graph data collected in year 2015. Median, upper and lower quartiles are represented. Points represent outliers.

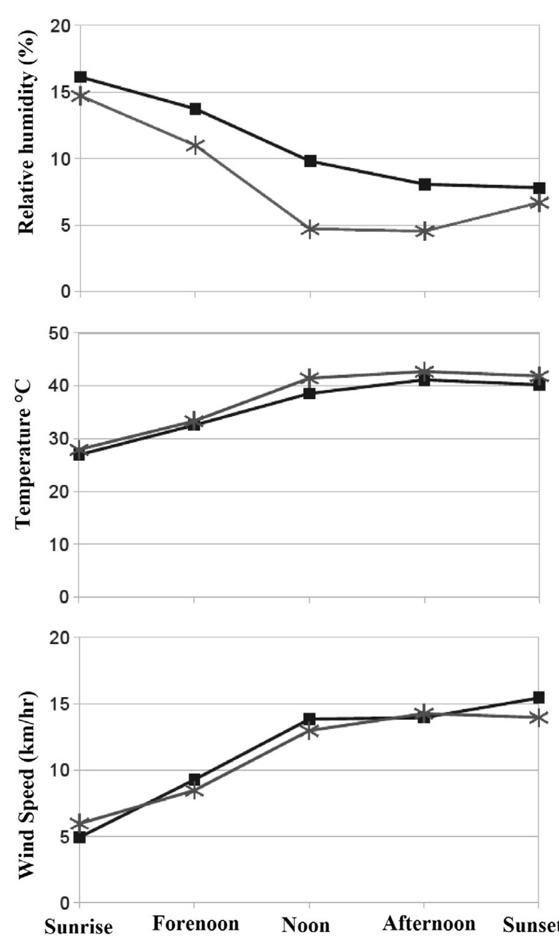

Fig. 5. Distribution of relative humidity, temperature and wind along the day, during the two field season in central Saudi Arabia (2013: black boxes; 2015: grey stars).

Table 1
Averages of total soluble solids (TSS) in different months in *Ziziphus nummularia* flowers and average temperature and relative humidity in central Saudi Arabia.

	Average TSS (μg)		Average temperature (°C)		Average relative humidity (%)	
	2013	2015	2013	2015	2013	2015
June	110.8	238.8	37.0	37.6	9.8	7.5
July	176.9	218.6	37.6	39.1	9.9	7.1
August	176.6	217.8	36.7	38.6	10.4	7.3
September	161.5	238.0	34.0	34.7	13.6	11.5
October	132.0	131.2	30.6	30.3	11.6	12.0

Fig. 2, shows the differences between the two years' data for each category as overall number of flowers without nectar and their monthly trends. In 1D flowers, we recorded an increase in nectar-empty flowers from 2013 (10.9%) to 2015 (13.1%). However, the seasonal trend was similar for both years with peaks of empty-flowers in July and September. On the contrary, for 2D flowers, we observed a decreasing tendency from 2013 (38.9%) to 2015 (27.4%). Along different months, in 2013 we observed the highest number of empty flowers in July, constantly decreasing afterwards. In 2015, the trend was slightly different with the highest number of empty flowers distributed among July, August, and September. The described differences were obtained from bagged flowers. Therefore, even among 1D flowers, there were flowers that did not produce nectar, and the percentage was significant. The absence of nectar resulted not only from insect visits as other factors often reported such as re-absorption and evaporation caused by differences in weather conditions (Búrquez and Corbet, 1991; Pacini and Nepi, 2007). Previous work of Alqarni (2015) already found that nectar is extremely concentrated in this species. We suggest that physiological limits and individual variability may influence nectar production of this species. In previous studies, flowers without nectar were not treated separately. Nevertheless, they are expected to influence bee behaviour. In fact, bees approach flowers but may reject them once they get very close (Howell and Alarcón, 2007), possibly being able to assess the absence of nectar (Corbet et al., 1984; Stout and Goulson, 2002). Rejecting behaviour has been recorded in different plant species flowers (Duffield et al., 1993). Flowers without nectar should be acknowledged separately and investigated from a physiological point of view.

In those flower that secret nectar, the amount was variable. TSS quantity was very different between the two categories of flowers in both years (2013 – Mann–Whitney $U = 8554.50$, $n_1 = 334$, $n_2 = 227$, $p < 0.0001$; 2015 – Mann–Whitney $U = 6733$, $n_1 = 304$, $n_2 = 254$, $p < 0.0001$; (Fig. 3). In 1D flowers, the daily TSS content was ranging from 2 to 966 μg (skewness 2,133), while in 2D flowers the range was 1–623 μg (skewness 10.185). Median of nectar TSS amount of 2D flowers in the two years ranged from 7 to 28 μg, while in 1D flowers it ranged from 14 to 409 μg. Nectar increases the chances of a flower to be visited by an effective pollinator attracted to its content. Nectar presence in 2D flowers may be related to the duration of stigma receptivity. Attracting bees to flowers with anthers already depleted may still valuable for depositing pollen previously collected from 1D flowers on a receptive stigma.

Focussing the attention on 1D flowers that contain significant amount of nectar, we found the same flow in TSS quantity along the day, in 2013 and 2015. TSS production was low at sunrise and forenoon, but it considerably increased at noon reaching its peak during the afternoon (Fig. 4). Finally, it decreased again by sunset; differences along daytime were significant (2013 – Kruskal Wallis, Chi Square = 172.30, df = 4, $p < 0.0001$; 2015 – Kruskal Wallis, Chi Square = 202.145, df = 4, $p < 0.0001$). The two years data showed the same flow but averages were different, being higher especially during noon and afternoon of 2015 (noon $p < 0.0001$; afternoon $p < 0.0001$). Nectar increase during the afternoon hours has been already recorded in other plant species (Schmidt et al., 2015; Nuru et al., 2015; Giovanetti et al., 2015) and bees have been recorded modulating their foraging behaviour to collect this resource after having collected pollen in the morning (Giovanetti and Lasso, 2005; Giovanetti et al., 2015).

The increased concentration of nectar during 2015 is very interesting, since it quite doubled the values recorded in 2013. Understanding what causes these variations may help in predicting honey production of a given year. Weather conditions (Fig. 5) may influence nectar production, especially its water content by inducing evaporation of the aqueous part of the solution, and consequently concentration of sugars. During the day, temperature increased from sunrise to noon, then continued around 40 °C during the afternoon till sunset. Wind showed the same pattern, increasing till noon and then being constant. On the contrary, relative humidity decreased constantly during the day; the decrease being more crucial in 2015. RH seems the more crucial variable to explain differences observed in TSS amount in 1D flowers. In fact, significant differences in RH emerged between years, only at noon and afternoon (noon, $p < 0.0001$; afternoon $p < 0.0001$). Moreover, TSS amount was significantly correlated with RH ($r = -0.61$, $p > 0.05$), but not correlated to temperature ($r = +0.36$, $p < 0.05$) and wind speed ($r = -0.14$, $p > 0.05$). Alqarni (2015) recorded, on the same species, an earlier peak of nectar concentration at noon but also different in daily values of RH. TSS values is expected to be related to RH. In fact, lower RH may correspond to a lower availability of water to dilute the nectar, therefore records of TSS should increase. Honey bee reported to prefer warmer flowers due to its less viscous nectar, independently from sugar concentration (Nicolson et al., 2013). Weather conditions in our study area may then sustain the collection of the highly concentrated nectar of *Z. nummularia*. This long term study showed that the TSS production has a seasonal trend from June to October, but the trend was strongly influenced by RH (Table 1). The peak nectar production was during July–August, while the minimum rate was during June. We observed a variation in all months of 2015, when actually RH monthly average values were lower. If recording lower RH, we may actually expect a higher solute concentration. Detailed observations on handling performance of honey bees on flowers at different times of the day and with different weather conditions are needed to further evaluate the appreciation of these aspects of plant-pollinator interaction, and consequently predict future income from the sector of beekeeping.

4. Conclusion

We demonstrated how the abundance of nectar could be predicted, first by flower age: in fact, we found that even if not all flowers may produce nectar, significantly higher quantities of nectar are found in 1-day old flowers. Second, relative humidity but not temperature or wind, is the only feature influencing TSS production, by a negative correlation.

The information presented above may help in predicting future yields of honey, and the economy correlated to its production. From this study, also a new topic emerged to be further investigated: the significance of empty flowers, the physiology correlated to their presence and the bees' response to their abundance.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group project No. RGP-189.

The authors appreciate provided accommodation facilities by the beekeepers; Mr. Faiz Ergaf and Mr. Mohamed Assodi during the field work.

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