



## RESEARCH ARTICLE - BEES

## The Ratio of Sunflower Pollens Foraged by *Apis mellifera* Is More Than That of *Apis cerana* Does During Sunflower Blooming

HP YANG, J SUN, P TANG, CS MA, SD LUO, J WU

Key Laboratory of Pollinating Insect Biology of the Ministry of Agriculture, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China

### Article History

#### Edited by

Solange Augusto, UFU, Brazil

Received	15 March 2019
Initial acceptance	02 October 2019
Final acceptance	30 March 2020
Publication date	30 June 2020

#### Keywords

Bias of pollens; Sunflower; Maize; Weeds; *Apis mellifera*; *Apis cerana*.

#### Corresponding author

Yang HuiPeng, Luo Shudong, and Wu Jie  
Key Laboratory of Pollinating Insect Biology  
of the Ministry of Agriculture  
Institute of Apicultural Research  
Chinese Academy of Agricultural Sciences  
No. 1 Beigou Xiangshan, Haidian District,  
100093, Beijing, China.  
E-Mail: yanghuiPeng@caas.cn /  
luoshudong@caas.cn / apis@vip.sina.com

### Abstract

Bias foraging of pollen is general in different pollinators since various nutrition demanding, co-evolution and interaction of insect-plant. To clarify the preference of pollen foraging during sunflower blooming, the pollen foraging behaviors of *Apis mellifera* Linnaeus and *Apis cerana* Fabricius were observed. Our results displayed that two summits of pollen foraging occurred in the morning before the ambient temperature climbed up to thirty-one degree centigrade and in the afternoon after the ambient temperature decreased below thirty-one degree centigrade, respectively. Notably, the first foraging summit of *Apis cerana* emerged one hour earlier than that of *Apis mellifera*. These results imply that *Apis mellifera* is less resistant to low temperature but more resistant to high temperature than *Apis cerana* does. The colonies were surrounded by sunflowers with sporadic weeds, while only few maize dispersed over two hundred meters away. However, no more than forty percent of total pollens foraged by *Apis mellifera* was from sunflower, and which was no more than twenty percent in *Apis cerana* group. These results suggest that sunflower pollens are not the prior choice for both honey bee species, while the ratio of sunflower pollens foraged by *Apis mellifera* is more than that of *Apis cerana* does.

### Introduction

Honey bees may visit a flower based on the floral color, size, patterning and social cues, and then were encouraged by the rewards of nectar or pollens (Orban & Plowright, 2014). However, the choice of nectar or pollens heavily depends on the colony nutritional deficiencies (Hendriksma & Shafir, 2016). Nectar is foraged as the major energy resource of adult bees and the pollens provide the protein, lipids, vitamins and minerals for both adult bees and larvae, while the realization of pollination by bees is mainly based on their foraging behavior (Brodshneider & Crailsheim, 2010).

The variations of available pollen resources are important for the health of honey bees due to the differences in nutrient composition (Di Pasquale et al., 2016). As the

generalist pollinators, honey bees play a dominant role in pollination service due to the widely foraging spectrum of plant species and the plentiful foragers of a single nest (Koski & Ashman, 2015).

The Eastern honeybee, *Apis cerana* Fabricius (*A. cerana*), is native to Asia and has been managed beekeeping at least 1700 years (Chen et al., 2017). There are more than 2 million *A. cerana* colonies kept in China (Chen et al., 2017). Compared to *Apis mellifera* Linnaeus (*A. mellifera*), *A. cerana* is better in stress-tolerance, such as scattered floral resources, extreme weather conditions, long flight duration, effective hygienic behaviors and cooperative group-level defenses (Park et al., 2015). Therefore, *A. cerana* takes charge of an essential complement of *A. mellifera* on pollination service for both wild plants and intensive crops (Park et al., 2015).



Large-scale single species plantation of sunflower (*Helianthus annuus*) or maize (*Zea mays*) destroys the habitats and results in decline of pollinators (Naug, 2009). Nowadays, the seed production of sunflower mainly depends on the pollination service of honey bees (Calderone, 2012), which are rewarded by both nectar and pollen (Greenleaf & Kremen, 2006). Meanwhile, the maize, as an anemophilous crop, flowers at the same period and can also provide pollen (Di Pasquale et al., 2016). Hence, the sunflower and the maize are main floral resources in July and August (Di Pasquale et al., 2016). However, the deficiency of histidine in maize pollens and phosphorus element in sunflower pollens, which are essential for honey bee health, results in dramatically reduced lifespan of workers (Di Pasquale et al., 2016; Filipiak et al., 2017). Furthermore, the clarification of nutritional requirements of different bee species will conduct the restoration of floral resource which develops diverse and nutritionally balanced plant communities (Vaudo et al., 2015).

In this current study, the colonies of *A. mellifera* and *A. cerana* were surrounded by intensive agrarian landscape of sunflower in Bayannur of Inner Mongolia autonomous region of China, where the sunflowers occupied the largest plant area and the most extensive range. (<http://www.bynr.gov.cn/BynChina/AboutByn/Famousbrandhigh/>). In order to demonstrate the bias pollen foraging behavior of these two honey bee species, the daily activity of pollen foraging and pollen preference have been observed during the flowering period.

## Materials and methods

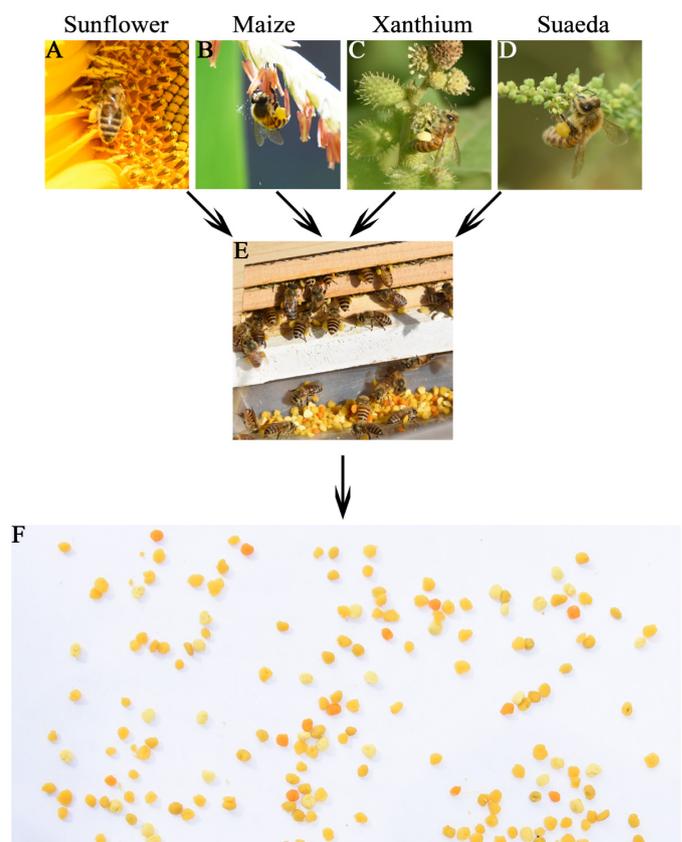
### Fields, surrounding vegetation and colonies

This study was performed in an agrarian environment in Bayannur of Inner Mongolia autonomous region of China (E107.40'38", N40.58'28" with altitude 1030 meters) from July 26th to August 26th of 2017 and 2018, where there were sunflower (92.9%) and maize (7.1%) crops during that time. Besides, few *Xanthium* and *Suaeda* distributing as weeds at the edge of the sunflower and maize fields were flowering. Due to the phenological consistency of these four plant species in the same habitat type, all pairwise comparisons were performed simultaneously. The colonies of *A. mellifera* were equalized to six frames of workers with one frame honey, while the colonies of *A. cerana* were equalized to three frames of workers with one frame honey. All the queens were half-year-old. For each bee species, five colonies were observed for foraging behavior. All the colonies were housed in standard Langstroth hives and shipped to the location two weeks before the sunflower blooming. The apiary was surrounded by sunflowers.

### Observation of foraging behavior

The number of bees returning with pollen pellets and the amount of pollen pellets were observed from 6:00

to 20:00 during 3 continuous sunny days at the full-bloom stage of sunflower, respectively. To record the number of workers returning with pollens, a camera was fixed in front of the entrance of each hive. Subsequently, the bees with pollen pellets were counted by eyes from the videos recorded by cameras at the first ten minutes of each hour. To obtain the pollen pellets collected by bees, pollen traps were set at the entrance of each colony (Fig 1). The captured pollen pellets were collected at each hour. And then, the pollen pellets were dispersed sufficiently so as to be recorded as photos for further counting (Fig 1). Compared to the pollen pellets from the maize, xanthium and suaeda (M.X.S.), the pollen pellets from sunflower exhibited distinct color (Fig 1). Consequently, the photos were digitally analyzed via Image Pro Plus software (Version 6.0) for the distinguishing and counting the sunflower pollen pellets from the M.X.S.. Meanwhile, the ambient temperature and humidity were recorded simultaneously by automatic recording meter (Eliteck RH-4HC).



**Fig 1.** Pollen foraging from sunflower (A), maize (B), xanthium (C) and suaeda (D) by honey bees. Pollen pellets collection via pollen trap (E) and recording via camera (F).

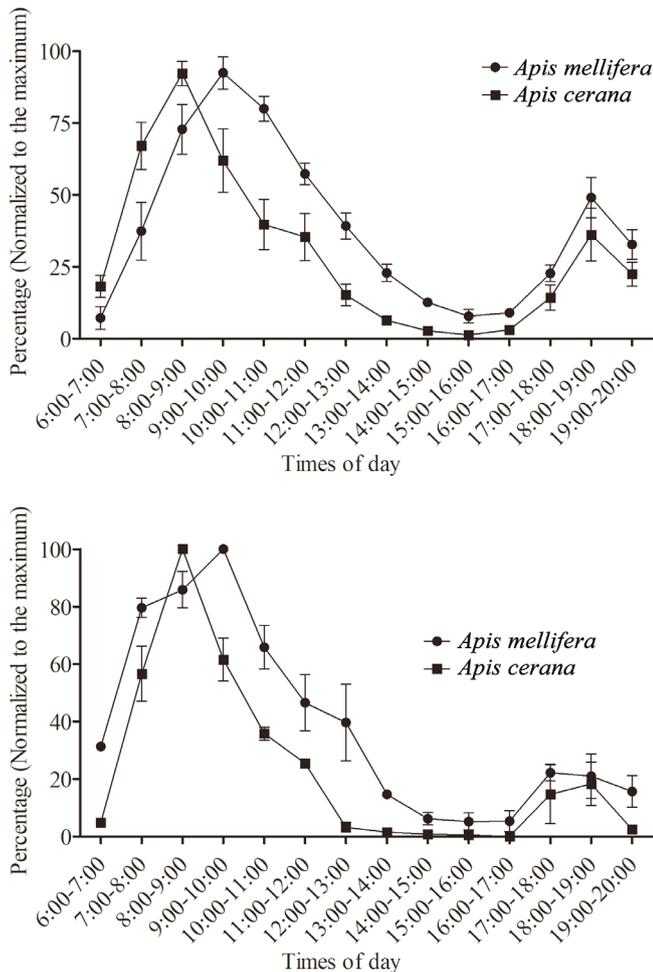
## Statistics

The data was analyzed using the software Prism (GraphPad Software, San Diego, CA), and all data was expressed as the mean  $\pm$  SE (Standard error). The results were analyzed using a two-way analysis of variance (ANOVA), followed by a statistical significance determination using Student's t-test.

**Results**

*Daily activity of honey bees*

The daily activity of *A. mellifera* and *A. cerana* was detected firstly. The number of workers returning with pollen pellets displayed that two summits occurred in the morning



**Fig 2.** The daily activity of honey bee foraging by worker counting (A) and pollen pellet counting (B). The ratio has been normalized to the maximum, respectively. Five colonies for three continuous sunny days for each bee species.

and afternoon, respectively. The first pollen foraging summit of *A. cerana* happened between 8 and 10 o'clock, while it was one hour later in *A. mellifera*. And then, the foraging behavior decreased sharply until another summit occurred between 17 and 19 o'clock (Fig 2A). Meanwhile, the amount of pollen pellets collected by pollen traps exhibited the same tendency (Fig 2B). During the observation period, the lowest temperature (13 degree centigrade) and highest humidity (94%) records happened at 6 o'clock, while the highest temperature record (36 degree centigrade) emerged at 12 o'clock and the lowest humidity record (23%) occurred at 15 o'clock (Fig 3).

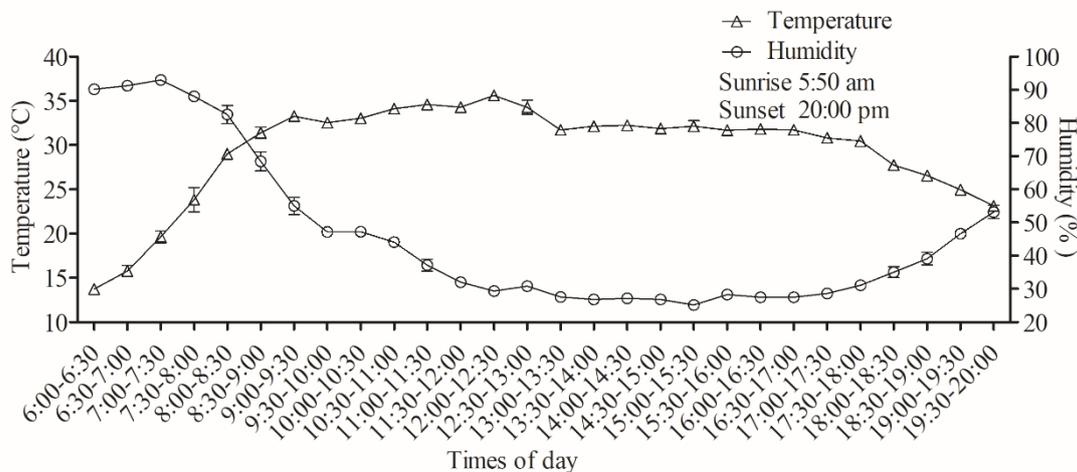
**Bias of pollen foraging between *A. mellifera* and *A. cerana***

As to the source of pollens, the ratio of sunflower pollen pellets were less than that of M.X.S. pollen pellets in both bee species (Fig 4A). Nevertheless, the ratio of sunflower pollens in *A. mellifera* was no more than forty percent, and which was no more than twenty percent in *A. cerana* (Fig 4A). For each species, *A. mellifera* foraged more sunflower pollens before 9 o'clock and post 15 o'clock, while they foraged more M.X.S. pollens between 9 o'clock to 15 o'clock (Fig 4B). In contrast, *A. cerana* foraged M.X.S. pollens more than that of sunflower during the entire observation period (Fig 4B).

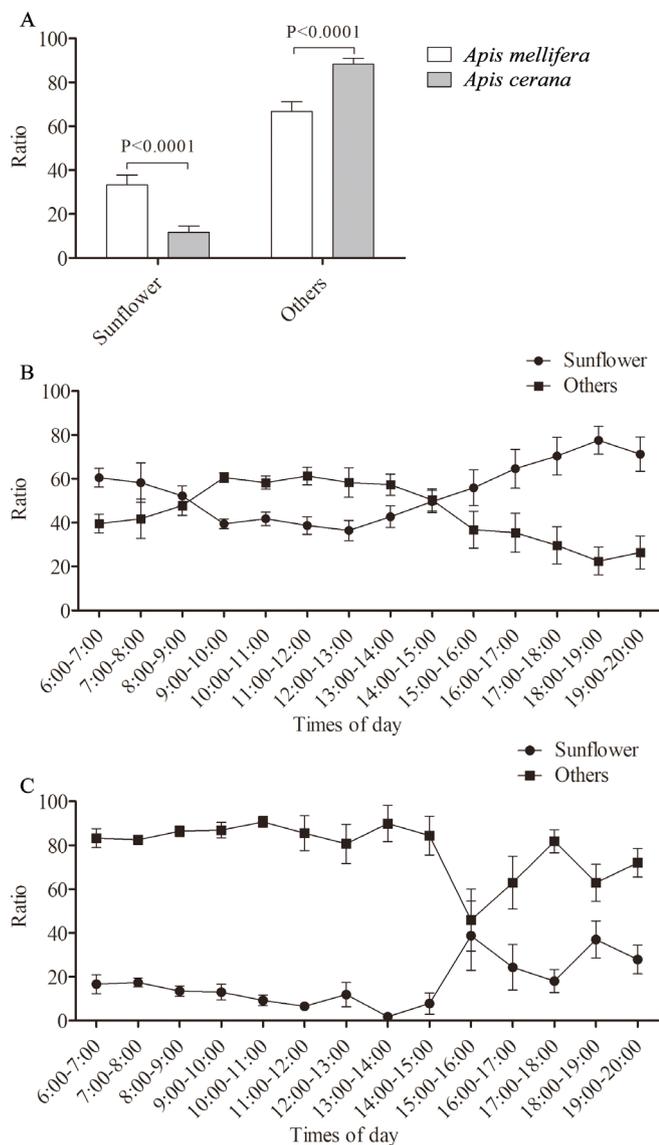
**Discussion**

For bee species, the foraging of pollen and nectar is important for the colony development, while the behaviors are affected by floral resources and ambient temperature (Aleixo et al., 2017). In the current study, the distinct differences of *A. mellifera* and *A. cerana* in foraging behavior and bias of pollens have been exhibited during the period of sunflower blooming with M.X.S as minor pollen resources and distributing sporadically.

Previously study shows that *A. cerana* starts foraging and reaches the peak of foraging activity earlier and at lower ambient temperatures than *A. mellifera* does, since *A. mellifera* demands higher thoracic temperature to forage than



**Fig 3.** The range of temperature and humidity of the apiary from sunrise to sunset for three continuous sunny days.



**Fig 4.** The ratio of pollen pellets foraged by workers for an entire day (A) and time sharing ratio of pollen pellets foraged by *A. mellifera* (B) and *A. cerana* (C). Five colonies for three continuous sunny days for each bee species.

*A. cerana* does (Tan et al., 2012). In this study, the increasing of foraging behavior was accompanied with the increasing of temperature, and the first summit in *A. cerana* happened one hour earlier than that of *A. mellifera*. However, the foraging behavior decreased sharply when the temperature was above 31 degree centigrade, and then a second foraging summit occurred in the later afternoon when the temperature went below 31 degree centigrade. What was more, the foraging behavior of *A. cerana* decreased earlier than that of *A. mellifera*. These results indicate that the *A. mellifera* is more tolerant to high temperature than *A. cerana* does.

Honey bees will weigh against the costs of time and energy with the gain of food (Couvillon et al., 2015; Danner et al., 2016). Although the colonies of both *A. mellifera* and *A. cerana* were surrounded by mass flowering sunflowers and only few other flowering plants distributed sporadically

in this current study, our results showed that more M.X.S. pollens were foraged. This phenomenon indicates that it is necessary for honey bees to forage M.X.S. pollens in spite of more time and energy demanded.

The behaviors of foragers are mainly driven by the colony nutritional deficiencies so as to maintain the health of colonies (Leonhardt & Blüthgen, 2012; Hendriksma & Shafir, 2016). Sunflower and maize are dominant pollen resource plants in summer, but their pollens are poorly nutrient for honey bees since the lower protein and essential amino acids content (Nicolson & Human, 2013; Di Pasquale et al., 2016). Even though, *A. mellifera* foraging more pollens of maize (Di Pasquale et al., 2016). Our results showed that both *A. mellifera* and *A. cerana* foraged more pollens of M.X.S. than that of sunflower, while *A. cerana* preferred foraging M.X.S. pollens more than *A. mellifera* did. Hence, the bias foraging of pollens might be due to the serious deficiencies of nutrition.

### Acknowledgements

This work was supported financially by Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2017-IAR) and the China Agriculture Research System (CARS-45). Besides, the authors would like to thanks the Croplife Asia for partially financial support.

### Authors' Contributions

Y.H.P., L.S.D. and W. J. conceived the study; Y.H.P., S.J., T.P., M.C.S., L.S.D. performed the experiments; Y.H.P. and L.S.D. analyzed the data and wrote this paper.

### References

- Aleixo, K.P., Menezes, C., Imperatriz Fonseca, V.L. & da Silva, C.I. (2017). Seasonal availability of floral resources and ambient temperature shape stingless bee foraging behavior (*Scaptotrigona aff. depilis*). *Apidologie*, 48: 117-127. doi: 10.1007/s13592-016-0456-4.
- Brodtschneider, R. & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, 41: 278-294. doi: 10.1051/apido/2010012.
- Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. *PLoS ONE*, 7: e37235. doi: 10.1371/journal.pone.0037235.
- Chen, C., Liu, Z., Luo, Y., Xu, Z., Wang, S., Zhang, X., Dai, R., Gao, J., Chen, X., Guo, H., Wang, H., Tang, J. & Shi, W. (2017). Managed honeybee colony losses of the Eastern honeybee (*Apis cerana*) in China (2011–2014). *Apidologie*, 48: 692-702. doi: 10.1007/s13592-017-0514-6.
- Couvillon, M. J., Riddell Pearce, F. C., Acceleton, C., Fensome, K. A., Quah, S. K. L., Taylor, E. L. & Ratnieks, F. L. W. (2015).

- Honey bee foraging distance depends on month and forage type. *Apidologie*, 46: 61-70. doi: 10.1007/s13592-014-0302-5.
- Danner, N., Molitor, A. M., Schiele, S., Härtel, S. & Steffan-Dewenter, I. (2016). Season and landscape composition affect pollen foraging distances and habitat use of honey bees. *Ecological Applications*, 26: 1920-1929. doi: 10.1890/15-1840.1.
- Di Pasquale, G., Alaux, C., Le Conte, Y., Odoux, J.-F., Pioz, M., Vaissière, B. E., Belzunces, L. P. & Decourtye, A. (2016). Variations in the availability of pollen resources affect honey bee health. *PLoS ONE*, 11: e0162818. doi: 10.1371/journal.pone.0162818.
- Filipiak, M., Kuszewska, K., Asselman, M., Denisow, B., Stawiarz, E., Woyciechowski, M. & Weiner, J. (2017). Ecological stoichiometry of the honeybee: Pollen diversity and adequate species composition are needed to mitigate limitations imposed on the growth and development of bees by pollen quality. *PLoS ONE*, 12: e0183236. doi: 10.1371/journal.pone.0183236.
- Greenleaf, S. S. & Kremen, C. (2006). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences*, 103: 13890-13895. doi: 10.1073/pnas.0600929103.
- Hendriksma, H. P. & Shafir, S. (2016). Honey bee foragers balance colony nutritional deficiencies. *Behavioral Ecology and Sociobiology*, 70: 509-517. doi: 10.1007/s00265-016-2067-5.
- Koski, M. H. & Ashman, T.-L. (2015). An altitudinal cline in UV floral pattern corresponds with a behavioral change of a generalist pollinator assemblage. *Ecology*, 96: 3343-3353. doi: 10.1890/15-0242.1.
- Leonhardt, S.D. & Blüthgen, N. (2012). The same, but different: pollen foraging in honeybee and bumblebee colonies. *Apidologie*, 43: 449-464. doi: 10.1007/s13592-011-0112-y.
- Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biological Conservation*, 142: 2369-2372. doi: 10.1016/j.biocon.2009.04.007.
- Nicolson, S. W. & Human, H. (2013). Chemical composition of the 'low quality' pollen of sunflower (*Helianthus annuus*, Asteraceae). *Apidologie*, 44: 144-152. doi: 10.1007/s13592-012-0166-5.
- Orban, L. L. & Plowright, C. M. (2014). Getting to the start line: how bumblebees and honeybees are visually guided towards their first floral contact. *Insectes Sociaux*, 61: 325-336. doi: 10.1007/s00040-014-0366-2.
- Park, D., Jung, J. W., Choi, B.-S., Jayakodi, M., Lee, J., Lim, J., Yu, Y., Choi, Y.-S., Lee, M.-L., Park, Y., Choi, I.-Y., Yang, T.-J., Edwards, O. R., Nah, G. & Kwon, H. W. (2015). Uncovering the novel characteristics of Asian honey bee, *Apis cerana*, by whole genome sequencing. *BMC Genomics*, 16: 1. doi: 10.1186/1471-2164-16-1.
- Tan, K., Yang, S., Wang, Z.-W., Radloff, S. E. & Oldroyd, B. P. (2012). Differences in foraging and broodnest temperature in the honey bees *Apis cerana* and *A. mellifera*. *Apidologie*, 43: 618-623. doi: 10.1007/s13592-012-0136-y.
- Vaudo, A. D., Tooker, J. F., Grozinger, C. M. & Patch, H. M. (2015). Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*, 10: 133-141. doi: 10.1016/j.cois.2015.05.008.

