4.2 Evaluating the feasibility of using the red mason bee (*Osmia bicornis* L.) in different experimental setups

Anke C. Dietzsch, Nadine Kunz, Ina P. Wirtz, Malte Frommberger, Jens Pistorius

Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Crop Protection in Field Crops & Grassland, Messeweg 11-12, 38104 Braunschweig, Germany
corresponding author: phone +49 531 299 4518, email anke.dietzsch@jki.bund.de

Abstract

Background: Evaluating hazards of pesticides to beneficial insects has become very important for the assessment and registration of pesticides. Test methods for honeybees are well established in the laboratory, under semi-field and field conditions. However, experiences in using other pollinators as model species are limited. Here we present results of various experiments on the red mason bee (*Osmia bicornis* L.), a solitary, commercially used bee species. The aim was to compare methodologies, to assess test parameters, and to evaluate the feasibility of using *O. bicornis* in late season, when this bee species would have already finished its life cycle under natural conditions.

Results: Hatching times and hatching rates varied depending on temperature and season. Provisioning and reproduction of *O. bicornis* were very variable, weather-dependent and not always reliably reproducible between seasons. They were higher in early than in late season in the field. In late season cardboard tubes showed greater cell production than wooden boards.

Conclusion: *O. bicornis* is a good study system under semi-field and field conditions: cocoons are easy to handle, and to monitor. Since hatching rate and cell production decreased over time, experiments are most recommended in early to mid season. Cardboard tubes can be used as standardised, inexpensive nesting devices. However, they do not allow continuous observation and pollen sampling, and involve time-consuming handling in the laboratory. Our experiment on nest material was conducted in late season and may not mirror conditions in spring and early summer.

Key words

Solitary bees, field experiments, semi-field experiments, reproduction, hatching, nesting

Introduction

Evaluating hazards of pesticides to beneficial insects has become more and more important for the assessment and registration of pesticides at both national and EU levels. Test methods for honeybees have been well established and have not only been applied in the laboratory but also under semi-field and field conditions.[1] However, experiences in using other commercially available pollinators as model species in different experimental setups are limited [but e.g. 2] and so far no guidelines are available. While honeybees are eusocial insects that form perennial colonies with many thousand individuals and can be repeatedly sampled at different seasons, most other bee species display small numbers of individuals per population, short periods of seasonal activity and restricted food preferences, which may be a challenge for using them in laboratory or field trials.

A solitary bee species that is well suited for experimental trials is the red mason bee, *Osmia bicornis* L. This species does not only reproduce under both laboratory and (semi-)field conditions [cf. e.g. 3,4] but it is also commercially available. The aim of this study was to compare methodologies in handling individuals of this bee species under semi-field and field conditions, and to evaluate test procedures in different experimental setups. Three questions were of particular interest:

- Can *O. bicornis* be used throughout the crop growing season in experimental trials?
- Are there any differences in hatching duration related to time of the season, which have to be considered in experimental setups?
- Which nesting material does *O. bicornis* prefer?

Experimental methods

Osmia bicornis L. is a univoltine bee species, whose main distribution range is Europe, but also parts of Northern Africa and the Middle East.[5] Individuals are 8-13 mm long and actively nesting from early April until mid June.[6] They exploit a wide range of flowering species.[7] Females prefer linear cavities as nests, which they divide in up to 20 compartments by mud walls. Cells are mainly provisioned with pollen. Larval development takes three to six weeks [8] and offspring hibernates as fully developed adult imagines in their cocoons.[9]

Studies were conducted between the beginning of April and end of July 2014. *O. bicornis* cocoons were purchased from a commercial breeder (WAB-Mauerbienenzucht, Germany) and stored at 4°C in the dark until used in experiments. Bee individuals were used in both field and semi-field experiments and were offered oilseed rape (*Brassica napus* L.) or phacelia (*Phacelia tanacetifolia* Benth.) as a foraging plant.

For the nesting material experiment, one nesting unit for emerging females either made from cardboard tubes or from milled wooden boards was installed in each of twelve 10m x 4m tunnels (Fig. 1) on a phacelia field on 18 July 2014. Each nesting unit was equipped with either 25 or 75 female and male cocoons, respectively, and provided three nesting cavities for each hatched female (i.e. either 75 or 225 nesting tubes per unit; n=3 tunnels per nesting material and unit size). Tunnels were placed in fields with the crop in full flower. Nesting units were covered with gauze after two to three weeks when the crop finished flowering.

After each experimental trial, all nesting units were covered with gauze and left at the field sites to facilitate undisturbed larval development for further three to four weeks. They were then transported to the laboratory where cardboard tubes were opened with an electrical saw. The number of produced cells and the number of cocoons were counted for each cardboard tube and wooden board unit, respectively.

a) b)

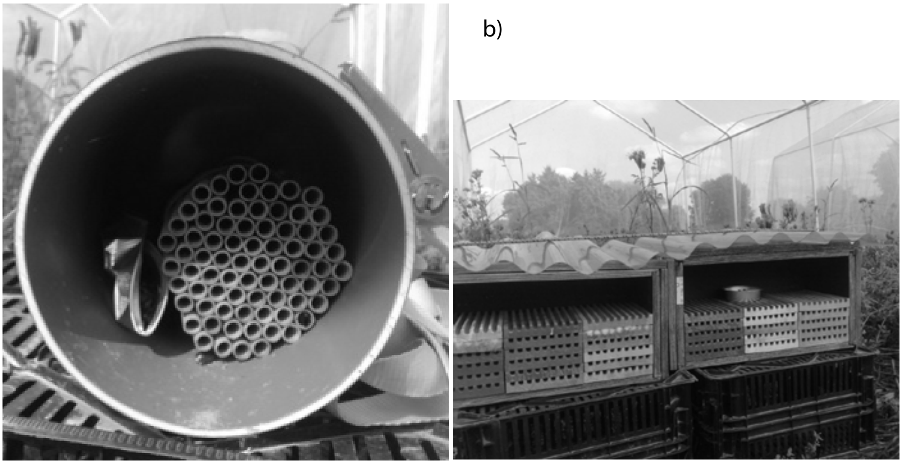

Figure 1 Different types of nesting units made from a) cardboard tubes or b) milled wood, each installed in one tunnel.

Hatching duration (defined as the period [in days] between exposing cocoons to experimental conditions and the hatching of 50% of bee individuals) and hatching rate (i.e. the ratio between successfully hatched individuals and total number of exposed cocoons x 100) were documented under field conditions in additional cardboard tube units equipped with 20 female cocoons each. Nesting units were continuously set up every two to three weeks throughout the season. Once a day, the number of newly emerged females was recorded. After each individual trial, all cocoons that failed to hatch were counted to estimate hatching rate.

Data were analysed in R [10] using generalized linear models (Poisson distribution, log-link function, with offset of number of hatched individuals) and likelihood ratio tests for model selection.

Results

Provisioning and reproduction of *O. bicornis* were very variable, weather-dependent and not always reliably reproducible between seasons. Hatching duration and hatching rates varied depending on temperature and season. Hatching rate was very high (up to 100%) at the beginning of the season but decreased in both sexes rapidly from the end of May onwards, falling as low as 5% and 4% for female and male individuals respectively by mid July (18 July 2014). Hatching duration decreased in both sexes at higher temperatures and later in the season with more than 50% of all male and female individuals hatched within one day by the end of June (26 June 2014).

Tube occupancy and production of cells was extremely low in the nest material experiment due to late season (Fig. 2a). Cardboard tubes held significantly more cells than wooden boards independently of the number of females per tunnel (Likelihood Ratio Test=103.1, $p<0.001$; Fig. 2a). An increase in the number of *Osmia* females increased cell production per female (Likelihood Ratio Test=7.0, $p<0.01$; Fig. 2a).

Early in the season, cell production rates in field trials were manifold higher than in late season; however, cell production in early season was also very variable (7.2 ±4.72 SD and 2.0 ±2.69 SD for early and late cell production respectively; Fig. 2b).

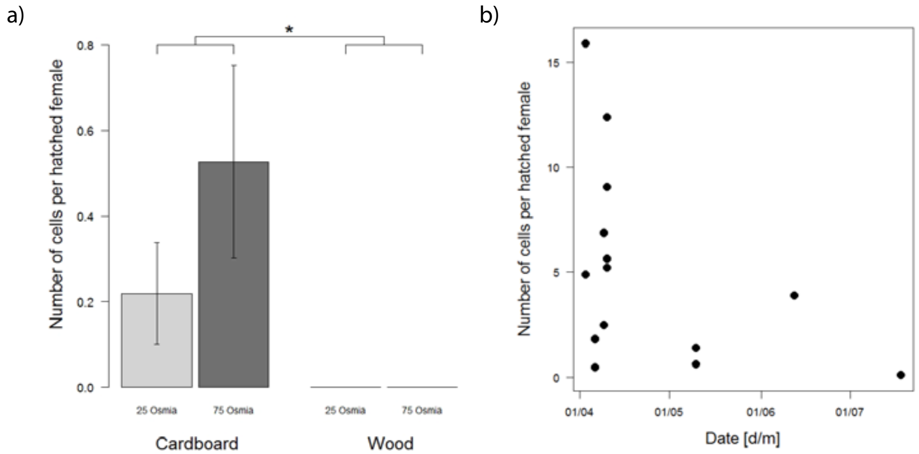

Figure 2 a) The mean number of cells [± standard error] built per hatched female in tunnels with either cardboard tube or wooden board nesting units. Each nesting unit was either stocked with 25 or 75 female (and male) O. bicornis cocoons. **b)** The number of cells per hatched female measured during various experimental field trials as a function of time.

Discussion and Conclusion

Like *Osmia* species in other geographical regions [11-13], *O. bicornis* appears to be a good study system under semi-field and field conditions in Central Europe: cocoons were easy to handle, to apply and to monitor, and pollen and nectar supply within tunnels were sufficient for the survival and reproduction of bees. Hatching rate and cell production greatly decreased over time making experiments carried out in July more unreliable. Experiments with *O. bicornis* are most recommended between May and mid June when >95% of individuals will hatch within 1-3 days given preferable weather conditions. Multiple replications of treatments are vital because observed variability within season is relatively high (mainly due to the sensitivity of *O. bicornis* to weather conditions).

In early season field trials showed higher nesting activity and cell provisioning than in late season, but early season occupancy was also very variable. Nest provisioning and the number of cells produced per female are a function of food availability [14] and flight activity, which depends in *O. bicornis* on weather conditions.[15] In the field all nesting units were placed close to a flowering crop which offered food in abundance. However, weather conditions were very changeable in early season, which may have affected cell production significantly. In contrast, equally low occupancy during late season may indicate general low activity levels of bees rather than unfavourable weather. Under natural conditions, *O. bicornis* actively forages until mid June.[7] Like in bumblebees whose mortality increases and egg laying rate decreases with prolonged hibernation [16], an artificial delay of *O. bicornis* hatching is likely to have caused the decrease in nesting activity in our study.

We found that cardboard tubes hosted a higher number of cells than wooden boards. Cardboard tubes can be a useful standardised nesting device in experiments where pollen sampling (for residue analysis) is mainly conducted at the end of the experiment. Such tubes are readily accepted by females and relatively inexpensive. However, they do not facilitate regular observations of cell provisioning within tubes during exposure. Continuous pollen sampling is also tedious to conduct: multiple nesting units have to be set up at the beginning of the trial and individually retrieved, cut open and replaced when a pollen sample is needed. In addition, handling of cardboard tubes in the laboratory is time-consuming. Other inexpensive nesting devices, like reed [15] or paraffinated paper straws [17], show similar problems. Wooden boards or blocks, which can be more easily disassembled for nest inspection [18] and pollen sampling, may reveal very low occupancy rates under certain conditions (e.g. semi-field, late season) as shown in this study. However, they may be more favourable than nest tubes made of plastic.[19]

Acceptance of a nesting device can vary between years [19] and season. Our experiment on nest material was conducted late in the season and may lead to different results during spring and early summer. Further tests on different nesting materials used at different seasons are needed to identify the most suitable nesting device to be standardized for pesticide testing purposes.

Acknowledgements

We thank members of the experimental field station of the JKI for their support. Parts of this study were funded by the Federal Ministry of Food and Agriculture.

References

1. EPPO, Environmental risk assessment scheme for plant protection products - Chapter 10: honeybees. *Bull. OEPP* **40**: 1-9 (2010).

2. Van der Steen JJM, Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie* **32**: 399-406 (2001).

3. Konrad R, Ferry N, Gatehouse AMR and Babendreier D, Potential effects of oilseed rape expressing oryzacystatin-1 (OC-1) and of purified insecticidal proteins on larvae of the solitary bee *Osmia bicornis*. *PLoS ONE* **3**: e2664 (2008).

4. Sandrock C, Tanadini LG, Pettis JS, Biesmeijer JC, Potts SG and Neumann P, Sublethal neonicotinoid insecticide exposure reduces solitary bee reproductive success. *Agricultural and Forest Entomology* **16**: 119-128 (2014).

5. The Global Biodiversity Information Facility, GBIF Backbone Taxonomy. http://www.gbif.org/species/5039314 [accessed on 8 April 2015] (2013).

6. Müller A, Amiet F and Krebs A, *Bienen: Mitteleuropäische Gattungen, Lebensweise, Beobachtung*, Naturbuch Verlag, München (1997).

7. Westrich P, *Die Wildbienen Baden-Württembergs*, Verlag Eugen Ulmer, Stuttgart (1989).

8. Tasei JN, Observations sur le développement d'*Osmia cornuta* Latr. et *Osmia rufa* L. (Hymenoptera Megachilidae). *Apidologie* **4**: 295-315 (1973).

9. Raw A, The biology of the solitary bee *Osmia rufa* (L.) (Megachilidae). *Transactions of the Royal Entomological Society of London* **124**: 213-229 (1972).

10. R Core Team, *R: a language and environment for statistical computing, R version 3.0.2*, R Foundation for Statistical Computing, Vienna, Austria (2013).

11. Bosch J and Kemp WP, Developing and establishing bee species as crop pollinators: the example of *Osmia spp.* (Hymenoptera: Megachilidae) and fruit trees. *Bull. Entomol. Res.* **92**: 3-16 (2002).

12. Abbott VA, Nadeau JL, Higo HA and Winston ML, Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* **101**: 784-796 (2008).

13. Biddinger DJ, Robertson JL, Mullin C, Frazier J, Ashcraft S, Rajotte EG, Joshi NK and Vaughn M, Comparative toxicities and synergism of apple orchard pesticide to *Apis mellifera* (L.) and *Osmia cornifrons* (Radoszkowski). *PLoS ONE* **8**: e72587 (2013).

14. Goodell K, Food availability affects *Osmia pumila* (Hymenoptera: Megachilidae) foraging, reproduction, and brood parasitism. *Oecologia* **137**: 160-160 (2003).

15. Bak B, Wilde J and Bratkowski J, The monitoring of the flight activity of the Red Mason Bee (*Osmia rufa* L.). *Acta Biologica Universitatis Daugavpiliensis* **3**: 97-100 (2003).

16. Gosterit A and Gurel F, Effect of different diapause regimes on survival and colony development in the bumble bee, *Bombus terrestris*. *Journal of Apicultural Research* **48**: 279-283 (2009).

17. Bosch J, Improvement of field management of *Osmia cornuta (*Latreille) (Hymenoptera, Megachilidae) to pollinate almond. *Apidologie* **25**: 71-83 (1994).

18. Bosch J and Kemp WP, *How to Manage the Blue Orchard Bee as an Orchard Pollinator*, Sustainable Agriculture Network, Beltsville, MD (2001).

19. Wilkaniec Z and Giejdasz K, Suitability of nesting substrates for the cavity-nesting bee *Osmia rufa*. *Journal of Apicultural Research* **42**: 29-31 (2003).