4.14 Experimental designs for field and semi-field studies with solitary wild bees

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Abstract

The newly proposed EFSA risk assessment of plant protection products for pollinators includes for the first time not only honey bees but also non-*Apis* pollinators (OEPP/EPPO 2010, EFSA 2013). No official guidelines for standardized tests exist so far. We performed field and semi-field studies to evaluate suitable test designs and handling procedures for the test organisms. The objective of these studies was the development of a test system for trials under field- and semi-field conditions with the red mason bee *Osmia bicornis* L. (Hymenoptera: Megachilidae).

The trials were conducted in two different crops, winter oilseed rape (*Brassica napus*) and Phacelia (*Phacelia tanacetifolia*), with different nesting materials, test designs and release techniques.

Methods

Semi-Field: The semi-field studies were performed during flowering in winter oilseed rape in spring and in *Phacelia* in summer at two different field sites in Southern Germany. Gauze covered tents were set-up containing one nesting unit made up of several chipboard drawers in the middle of each tent. Two release rates (simple and double) were tested in each crop - one replicate per rate in winter oilseed rape and two replicates per release rate in *Phacelia* (Table 1). A toxic reference (spray application during the flight) with 1000 g a.i. dimethoate/ha was included in the test design for oil seed rape. For both studies the reproduction rate was obtained from observed cell production and nest occupation by females. The development of cell production was documented by photographic evaluation for the study in winter oilseed rape.

Table 1 Test design for semi-field studies (SR = simple release rate, DR = double release rate, ♀ = female, ♂ = male)

	Brassica napus	*Phacelia tanacetifolia*
number of tents	2	4
size of tent	40m²	40m²
release rate	simple: SR, 24♀ 48♂ double: DR, 48♀ 96♂	simple: SR, 48♀ 72♂ double: DR, 96♀ 144♂
hatching success	95%♀ 80%♂	90%♀ 89%♂
nesting material	chipboard units (100 nesting holes)	chipboard units (100 nesting holes)

Field: The field study was performed in *Phacelia* during summer at a field site located in Southern Germany. Nesting units with four different nesting materials were installed. Two replicates of each nesting material were tested with one release rate (77♀ / 54♂). Nesting materials tested were natural reed tubes, chipboard units, wooden drawer units (provided by the Red Beehive Company) and paper tube liners. The attractiveness of the different nesting materials was evaluated based on the (observed) number of nesting females.

Results

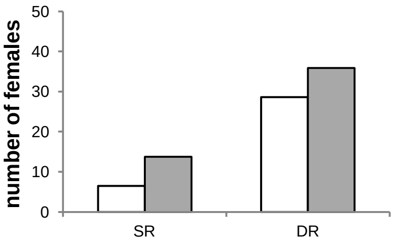

Figure 1 The mean number of nesting females of *Osmia bicornis* per release rate in both crops *Brassica napus* (white) and *Phacelia tanacetifolia* (grey). SR = simple release rate, DR = double release rate.

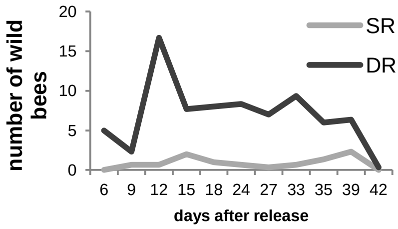

Figure 2 The total number of females of *Osmia bicornis* entering the cavities per fixed time period depending on the release rate in winter oil seed rape during the experimental phase (SR = simple release rate, DR = double release rate)

Semi-Field, *Nest Occupation:*The different release rates were evaluated based on the number of nesting females for both crops. As expected the double release rates resulted in a higher nest occupation in both trials (Fig.1). Daily observations showed a ratio for SR (simple release) / DR (double release) of 1/3.6 in winter oilseed rape and 1/2.3 in *Phacelia*.

Semi-Field, *Flight Activity:* As assumed the flight activity was about 3-4 times higher in the treatment with the double release rate (Fig. 2).

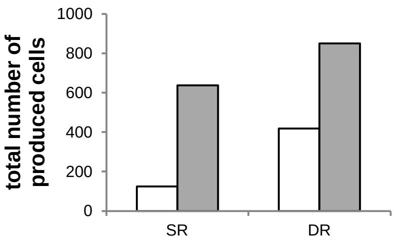

Figure 3 Total number of produced cells of *Osmia bicornis* depending on the release rate in both crops *Brassica napus* (white) and *Phacelia tanacetifolia* (grey). SR = simple release rate, DR = double release rate.

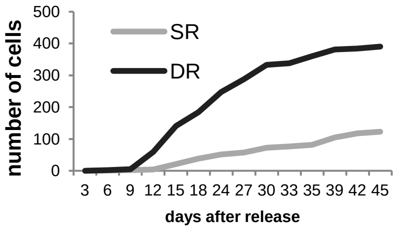

Figure 4 Number of produced cells of *Osmia bicornis* depending on the release rate in winter oil seed rape during the experimental phase (SR = simple release rate, DR = double release rate)

Semi field, *Cell Production:* The mean cell production was calculated from the total number of produced cells during the experimental field phase. As observed for the nest occupation, the cell production was higher for both crops with the double release rate (Fig.3).

The number of produced cells was rising until the end of the study in both treatments. The rate of increase was much higher (until approx. 4 weeks after release) in the treatment with double release rate compared to the single release rate. The rate of cell production was comparable in both release rates (Fig.4).

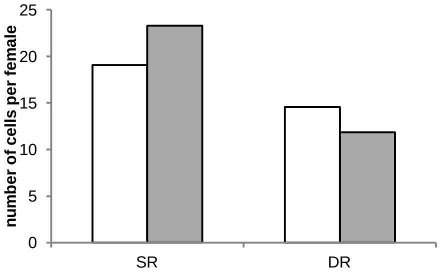

Figure 5 The mean number of produced cells per female of *Osmia bicornis* depending on the release rate in both crops *Brassica napus* (white) and *Phacelia tanacetifolia* (grey). SR = simple release rate, DR = double release rate.

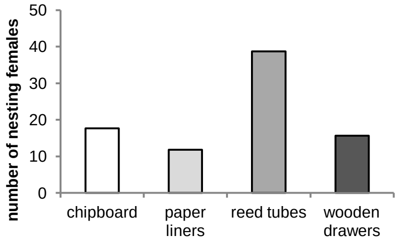

Figure 6 The mean number of nesting females of *Osmia bicornis* (per 100 nesting holes) per the nesting material

Semi field, *Reproduction:* To identify an ideal release rate for optimal cell production, the number of produced cells per nesting female was calculated. Interestingly, although the double release rate yielded larger total numbers of produced cells, the number of produced cells per female was higher for the simple release rate in both trials (Fig.5).

Semi field, *Toxic reference/sensibility:* The toxic reference showed a strong impact on the wild bees. No cell production at all was observed after exposure in the replicate treated with the toxic reference.

Field, *Attractiveness:* The evaluation of the mean numbers of nesting females gave the following order (with decreasing attractiveness) of the four nesting materials (Fig 6.):

Reed tubes > *Chipboard units* > *Wooden drawer units* > *Paper liners.* The nesting females of *Osmia bicornis* preferred the natural material of reed tubes followed by clipboard and wooden drawer units. The lowest attractiveness was observed for the paper liners.

Discussion and conclusions

Semi-Field Test Design: The results show that studies with the red mason bee *Osmia bicornis* L. are possible in both tested crops. Even a late start of studies in summer is possible when the cocoons are kept under constant conditions (cooled at 4°C) until release. In order to obtain good nest occupation rates and to yield a high number of produced cells for further observation, the higher (double) release rate is preferable. However, the reproduction rate per nesting female is higher in the simple release rate. As a consequence, two considerations should be mentioned: (1) competition because of dense nesting sites (Torchio, 1985), (2) competition for food resources due to a compacted bee / flower ratio (Bosch and Kemp 2001). These effects should be taken into account for identifying the ideal test design for semi-field studies.

Field Test Design: For field studies, a higher release rate has to be considered as the dispersal of females is much higher compared to a semi-field set-up. Regarding the preference of nesting materials, it seems that natural and more uneven nesting tubes (reed) are more attractive than other nesting materials (Wilkaniec and Giejdasz, 2003). In terms of practical handling of the nesting units during the assessment phase, the nesting material should be adapted to the objective of the studies. If observations of cell production over time are required, only chipboard and drawer systems seem to be appropriate.

Solitary bees like *Osmia bicornis* have specific life history traits and requirements for natural resources and show other reactions to stressors. The challenge is to develop a general test design for risk assessment with wild bees, considering the many influences in natural environments such as the varying availability of nesting and food resources, of material of nesting sites, of bee densities and sex ratio (Sedivy and Dorn 2014). Consequently future studies should take these factors into account.

References

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