3.9 Semi-field testing of the solitary bee *Osmia bicornis* (L., 1758) (Hymenoptera, Megachilidae) in flowering *Phacelia tanacetifolia* – Chances, improvements and limitations

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Abstract

Based on the proposed test design of the ICPPR non-*Apis* working group, a semi-field (tunnel) study was conducted with the solitary bee *Osmia bicornis* (L., 1758) in flowering *Phacelia* using tunnels. Untreated crop was used as a control, and the insect growth regulator fenoxycarb as a test item which is intended to be used as reference item for this study type. To improve the design and to enhance the informative value of such studies our method deviated in three points: placing the cocoons in the tubes of the nesting units, performing additional brood assessments in two to three day intervals and increasing the application rate of the test item.

Overall the results indicated that the proposed test design is suitable to perform studies on *O. bicornis* in *Phacelia* under semi-field conditions.Data on the reproduction performance, brood termination and hatching rate of the progenies show low variability between the replicates in both treatment groups. No impact on the flight activity, mortality, reproduction performance and hatching success of the progenies was observed, but an increased brood termination rate of larvae produced within the first days after application was recorded; in particular, placing the cocoons in the tubes lead to higher proportions of nesting established females. Moreover, due to the assessment of the cell production in 2 to 3 day intervals, it is possible to analyse time dependent effects on the reproductive performance, brood termination and hatching rate which can be expected by the decreasing exposure in the course of the study. And finally, it is shown that fenoxycarb is not a suitable reference item for such studies.

Keywords: Solitary bees, Osmia bicornis, higher tier testing, semi-field, improvements

Introduction

According to the 'EFSA Guidance Document on the risk assessment of plant protection on bees' (EFSA 2013), in addition to honeybees, it is now also necessary to consider bumble bees and solitary bees for the first time. However, suitable testing methods were missing not only for laboratory but also for semi-field and field testing. Regarding the semi-field exposure the ICPPR non-*Apis* working group developed a test design on the testing of solitary bee species, e.g. *Osmia* spec.during two workshops: in the spring of 2015 in Limburgerhof (ICPPR 2015) and in 2016 in Braunschweig (ICPPR 2016). Among other endpoints, the number of cells with eggs produced per female, the failure of such cells to develop (expressed as the 'brood termination rate', BTR) and the hatching rate of the progeny (F1-generation) from the cocoons were regarded as the key endpoints of these studies.

To evaluate the suitability of the test design, we performed a semi-field study in 2016 but deviated from the proposed test design in three points to improve the performance and enhance the informative value of the study: we placed the cocoons in the tubes of the nesting units, performed additional brood assessments and increased the application rate of the test item fenoxycarb which was intended to be used as reference item for this study type.

Material and Methods

Design

The layout of the semi-field study is based on the design proposed by ICPPR non-Apis working group (e.g. 90 m^2 of flowering Phacelia; untreated control, fenoxycarb as test item, each with four replicates; O. bicornis as test species; pre-application and exposure period: 10 days, each) with the following main differences:

- cocoons (38 female and 60 male cocoons per replicate) were provided in the tubes of the nesting units instead using release trays
- aim: to increase the number of nesting established females (nest occupation)
- assessments of cell production were performed in 2 to 3 day intervals during the exposure phase instead of one single assessment at the end of the exposure period aim: to address time-dependent exposure of the adults (i.e. cell production/female) and larvae (i.e. BTR, hatching rate of the progenies from cocoons in the subsequent spring) for non-systemic products during the consecutive test intervals
- the application rate of fenoxycarb was increased to 600 g a.s./ha (4-fold of registered rate)
  aim: to increase the BTRs as application rates of up to 350 g a.s./ha displayed BTRs < 50% (KNÄBE et al., 2016) which is insufficient for brood studies
- Assessments were done on
- the hatching success of the introduced cocoons on DAT (Days After Treatment)-9, -7, -4 and -2,
- the flight activity on the day of treatment (DAT 0) shortly before and 2 & 4 h after application as well as on DAT 1; recording of the number of female bees entering the nesting unit within 3 minutes time (assessments were done in duplicate)
- the cell production (i.e. complete cells = closed cells containg food and an egg) and the nesting females (nest occupation) on DAT -1, 2, 4, 7 and 9 in the evening after bee flight; the produced cells of each test interval were marked with a coloured marker on a transparaent sheet and counted
- the brood development on DAT 37 determining the number of larvae reaching the cocoon stage
- the hatching rate of progenies (F1-generation) from cocoons in spring 2017.

Key endpoints were the number of nesting females [n] in a certain test interval the cell production per nesting female [n]

$$n = \frac{\text{nC produced between DATx and DATx} + 1}{(\text{nF DATx} + \text{nF DATx} + 1)/2}$$

n = number of complete cells/female in a certain test interval

nC = number of complete cells; nF = number of nesting females

DATx = day x of the study; DATx +1 = subsequent assessment after DATx

Brood Termination Rate [%]

$$\text{BTR [\%]} = \frac{\text{nL that did not reach cocoon stage for nC produced between DATx and DATx} + 1}{\text{nC produced between DATx and DATx} + 1} * 100$$

BTR [%] = Brood Termination Rate in a certain test interval

nL = number of larvae; nC = number of complete cells; CO = cocoon stage

DATx = day x of the study; DATx +1 = subsequent assessment after DATx

Hatching rate [%]

$$\text{HR [\%]} = \frac{\text{nCO that hatched, produced between DATx and DATx} + 1}{\text{nCO produced between DATx and DATx} + 1} * 100$$

HR [%] = Hatching Rate of progenies from cocoons, produced in certain test interval and attributed to a certain sex

nCO = number of cocoons

DATx = day x of the study; DATx +1 = subsequent assessment after DATx

The endpoints were evaluated for each test interval (DAT -1 to 2, 2 to 4, 4 to 7, 7 to 9) and the overall test period.

Statistical evaluations

The data of nesting females and of reproduction were Log-transformed whereas those of the BTR and hatching were arcsin-square-root transformed; subsequently, data were examined for normal distribution (Shapiro-Wilk test) and homoscedasticity (Bartlett's test). The final evaluation was done using Student t-test ($p = 0.05$).

Determination of 'Minimum Detectable Differences' (MDD) as an indication of statistical power was done based on BROCK et al. (2015)

Results

- The mean hatching success of the females in the control and test item prior the test was 89% and 90%, respectively, and 94% and 95% for the males.
- Based on the number of hatched females, the mean nest occupation rate was 90% and 97% at DAT -1 in the control and test item group, respectively.
- The flight activity indicated that females were well exposed during the application and the day after with fenoxycarb having no impact on this endpoint (Fig. 1).
- The mean number of nesting females decreased in a comparable pattern in both treatment groups ($p > 0.05$ at each assessment day) indicating the absence of any test item related lethal effect (Fig. 2).
- Overall, 6.6% and 6.1% of all cells in the control and test item group were incomplete, *i.e.* with no eggs being present. Approximately half of them were built between DAT -1 and DAT 2.
- The mean production of complete cells/nesting female during each test interval and the overall exposure phase was on a similar level in both treatment groups and thus with no statistically significant differences present ($p > 0.05$) (Fig. 3). MDDs were determined to be 0.8 (=25.6%), 0.5 (=18.7%), 0.4 (=7.6%), 0.3 (31.0%) and 1.5 (=12.6%) cells/nesting female for the respective and the overall test period, respectively.
- The mean BTR was 3.1% for the overall test period and 9.3% in the control and test item group and statistically significantly different ($p < 0.05$) (Fig. 3). The mean BTR also statistically significantly increased in the test item group for cells produced between DAT -1 and 2 (18.3%), but not in the subsequent intervals. The MDDs amounted to 2.0% for the overall period and 7.0%, 4.1%, 3.8% and 5.9% for the respective test intervals.
- The overall mean hatching rate of the progenies in the control and test item group was 98.7% and 94.7% for the females, respectively, and 98.5% and 96.9% for the males, accordingly (Fig. 4). For the respective test interval.
- The hatching rates of the females varied between 94.9% and 100% in the control and between 82.5% and 100% in the test item group. For the males the rates were between 96.5% and 100% in the control and between 91.4% and 100% in the test item group. No statistically significant differences were observed; neither for the overall test period nor for the single test intervals ($p > 0.05$). MDDs amounted to be 8.6% and 4.8% for the females and males for the overall test period; for the respective test intervals MDDs were between 4.1% and 13.1% for the females and between 2.9% and 6.9% for the males.

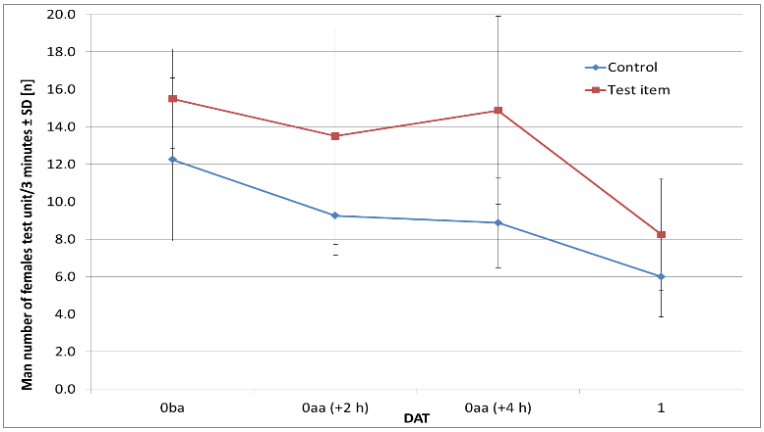

Fig. 14 Flight activity shortly before and after the application

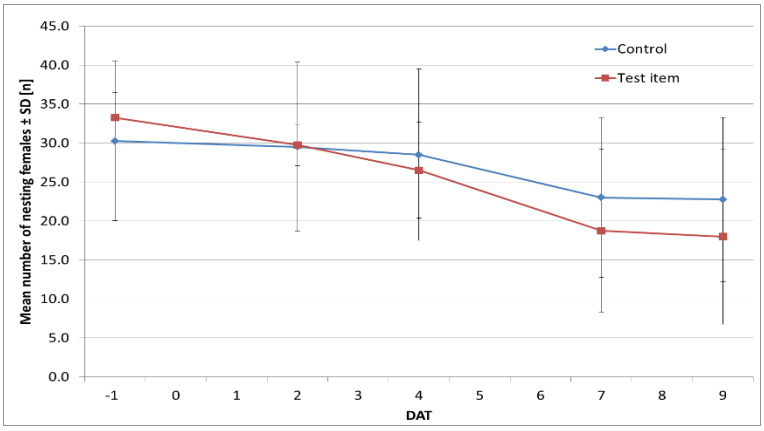

Fig. 15 Nesting activity in the course of the study

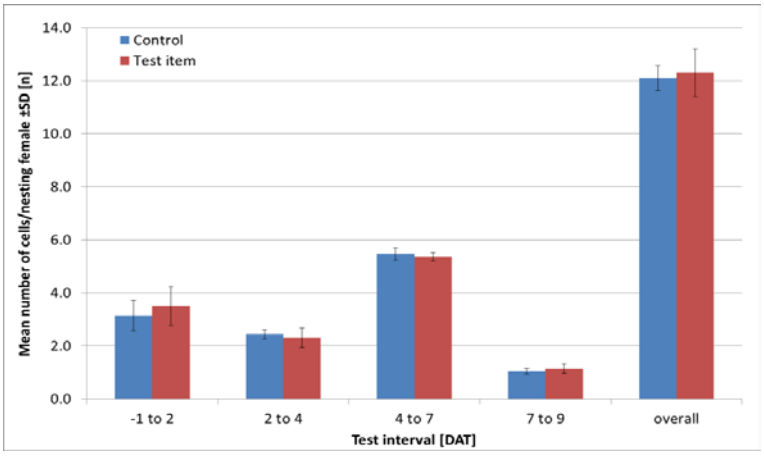

Fig. 16 Test interval-dependent and overall reproduction performance

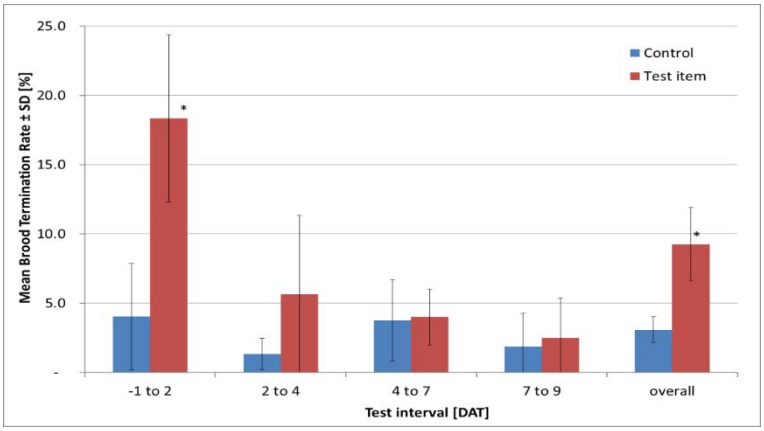

Fig. 17 Test interval-dependent and overall BTR (* = stat. sign. different, t-test, $p < 0.05$)

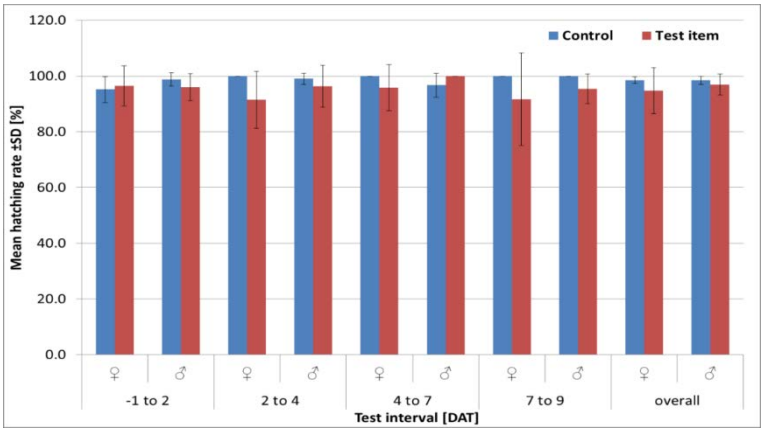

Fig. 18 Test interval-dependent and overall hatching rate

Discussion and Conclusion

The findings indicate that

- the proposed test design is principally suitable to perform studies on *O. bicornis* in *Phacelia* under semi-field conditions. Data on the reproduction performance, brood termination and hatching rate of the progenies in the subsequent spring shows low variability between the replicates in both treatment groups. Thus, even small differences in the endpoints can be detected.
- provision of cocoons in the tubes of the nesting units instead of using release trays lead to distinctly higher proportions of nesting females. This is compared to occupation rates on DAT -1 of approx. 72% to 85% and 73% (based on the number of hatched females) observed by KNÄBE et al. (2016) and KONDAGALA et al. (2016), respectively.
- the assessment of the cell production in 2 to 3 day intervals enables analysis of time-dependent effects on the reproductive performance per nesting female, brood termination and hatching rate. This is due to decreasing exposure throughout the course of the study (see Workshop on Pesticide Exposure Assessment Paradigm for non-*Apis* Bees 2017) and not only for the total period.

- in fact the BTRs observed for the overall test period and the first test interval in the test item group were statistically significantly increased compared to the control but nevertheless rather low for an intended reference item, even at the increased rate. Thus fenoxycarb is not a suitable reference item for such studies and therefore it is recommended to search for an alternative active ingredient which affects the larval development of *O. bicornis* more considerably.

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