Fenoxycarb, a suitable reference item in semi-field testing on the solitary bee *Osmia bicornis* (L., 1758) (Hymenoptera, Megachilidae)?

Johannes Lückmann, Christian Claßen, Oliver Mayer, Oliver Jakoby, Ribana Seliger
RIFCON GmbH, Goldbeckstr.13, 69493 Hirschberg, Germany, johannes.lueckmann@rifcon.de

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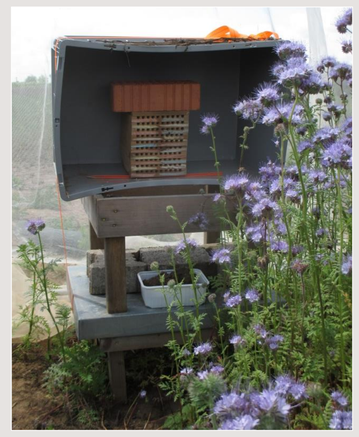
Introduction

According to the 'EFSA Guidance Document on the risk assessment of plant protection on bees' [1], not only honeybees but also bumble bees and solitary bees have to be considered for the first time. But for testing of solitary bees under laboratory, semi-field and field conditions no official test guideline exists. Regarding the semi-field exposure a design was developed by the ICPPR non-Apis working group, which is mainly based on the outcome of two workshops of this group in spring 2015 [2] and 2016 [3]. Next to others, the number of cells with eggs produced per female, the failure of such cells to reach the cocoon stage, 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 such studies.
One result of the studies from 2015 was that the used application rates of the reference item fenoxycarb, an insect growth regulator routinely used in brood studies on honeybees [4] [5], *i.e.* 150 and 350 g a.s./ha produced statistically significant increased BTRs, but which were below 50% for both rates [6]. Such low BTRs are not adequate to show the suitability of the test design. The reasons might have been that the test species *Osmia bicornis* (L., 1758) was not susceptible to fenoxycarb or fenoxycarb was under-dosed. Therefore, a study performed in 2016 investigated the effects of a higher rate.



Material and Methods

The semi-field study consisted of an untreated control and a test item (fenoxycarb) treatment group, each with four replicates, *i.e.* tunnels with 90 m² of flowering *Phacelia tanacetifolia*. Specimen of both sexes of *O. bicornis* were provided as cocoons in nesting units 10 days before the application to let them hatch and mate and to establish females in nesting activity. The application rate of fenoxycarb was 600 g a.s./ha which is four times the recommended rate. Bees were exposed to the treated crop for 10 days. Flight activity, *i.e.* number of female bees entering the nesting unit within 3 minutes, was assessed shortly before treatment, 2 and 4 hours after application as well on the day after to verify exposure. The number of nesting females and the number of cells produced was determined in the evening of DAT (Day After Treatment) -1 (day before treatment), DAT 2, 4, 7 and 9. The subsequent development was investigated on DAT 37, *i.e.* when all larvae would have reached the cocoon stage. Based on the number of cells produced and the number of larvae that did not reach this stage and thus displayed an unsuccessful development the BTRs for the control and test item group were determined for the entire test period as well as for the respective test intervals, *i.e*. DAT -1 to 2, 2 to 4, 4 to 7 and 7 to 9. The hatching success was determined in spring 2017 and related to the number of cocoons. For statistical analysis reproduction data were log- and BTR and hatching data were arcsin-square-root transformed, examined for normal distribution (Shapiro-Wilk test) and homoscedasticity (Bartlett's test) and finally evaluated using Student t-test. As an indication of the statistical power 'Minimum Detectable Differences' (MDD) were calculated to identify the difference between the means of the test item treatment and the control group that must at least exist to detect a statistically significant effect [7].

Results

The data of the flight activity indicated that bees were well exposed during the application and the day after and fenoxycarb had no impact on this endpoint (Table 1). Moreover, mean cell production per nesting female during the entire test period was on a similar level in both treatment groups and not statistically significant different (Table 1.). The absolute MDD was 1.5 cells/female which is 12.6% of the mean value of the control.

Table 1: Flight activity, reproduction success and hatching rate of *Osmia bicornis* under semi-field

Endpoint	Flight activity [no. of female entering the nesting unit / 3 minutes]								Reproduction [no. of cells / nesting female]		Hatching rate [%]			
DAT, timing	0bt		0at, +2 h		0at, +4 h		1, midday		Entire period		Entire period			
Treatment group	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Females		Males	
											Mean	± SD	Mean	± SD
Control	12.3	4.3	9.3	2.1	8.9	2.4	6.0	2.2	12.1	0.5	98.7	2.5	98.5	1.5
Test item	15.5	2.6	13.5	5.8	14.9	5.0	8.3	3.0	12.3	0.9	94.7	8.3	96.6	3.8

DAT = Day After Treatment, bt = before treatment, at = after treatment

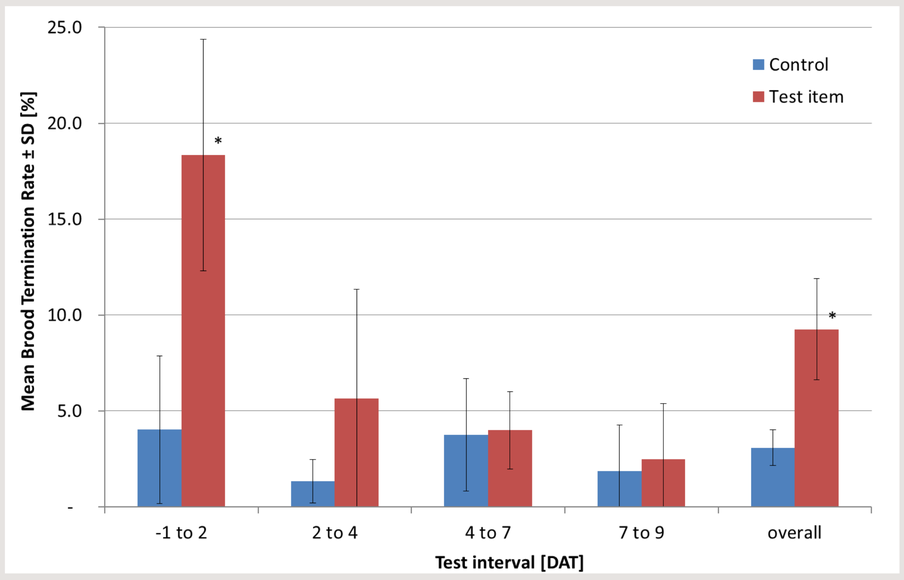
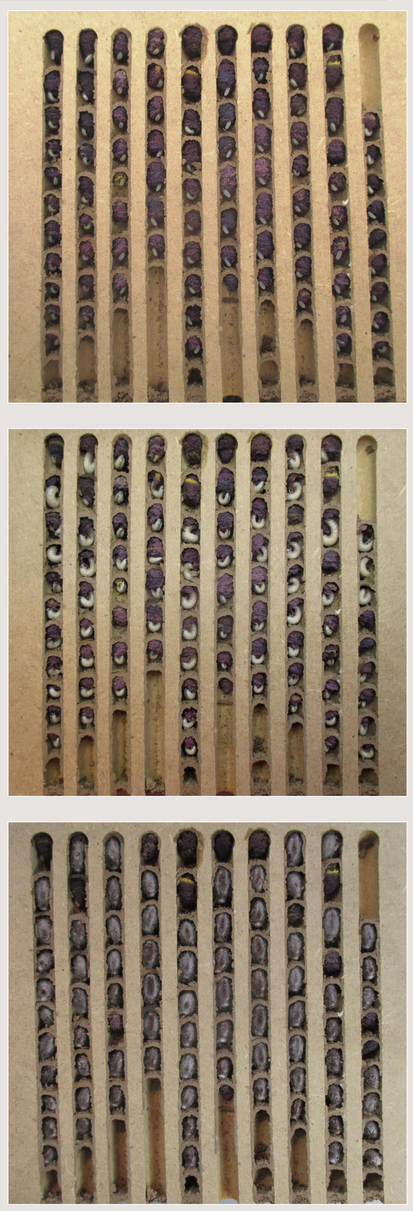


Figure 1: Overall and test interval-dependent brood termination rate
(* = stat. sign., t-test , $p < 0.05$)

The overall mean BTR in the control group was 3.1% and varied between 1.3% and 4.0% for the respective test intervals (Figure 1). In the test item group overall mean BTR was slightly increased and amounted to 9.3%. Taking into account the different test intervals mean BTR in the test item group was highest for cells produced between DAT -1 and DAT 2 (18.3%), subsequently decreased (5.6%) and was on the control level from DAT 4 onwards. Statistically significant higher BTRs were observed for the first testing interval and the overall period, whereas none were detected for the remaining intervals. The MDD analysis for the overall BTR showed that a nominal difference in the termination rates between the control and test item group means of at least 2.0 % was detectable. For the different test intervals, *i.e*. DAT -1 to DAT 2, DAT 2 to 4, DAT 4 to 7 and DAT 7 to 9 these differences were 7.0%, 4.1%, 3.8% and 5.9%, respectively. In contrast to the BTRs, no impact on the overall hatching rates and thus no statistically significant differences were observed (Table 1) with nominal MDDs of 7.5% for the females and 4.8% for the males.



Discussion & Conclusion

The findings (*i.e.* good reproduction success, low BTRs, high hatching rates in the control) indicate on the one hand that the proposed test design is suitable to perform studies on *O. bicornis* in *Phacelia* under semi-field conditions with data in both treatment groups showing low variability between the replicates. Thus, even small differences in the endpoints can be detected. Moreover, as in solitary bees the total provisions needed for larval development are collected over a short period of time [8] and BTR data indicate a decreasing exposure of the larvae to fenoxycarb residues in the course of the study it is recommended to assess the BTRs in two to three days intervals and not only for the total period.
On the other hand, in the test item group the overall BTR and the BTR observed in the first test interval were distinctly below 50%. This is rather low for a reference item, even at the fourfold application rate of this test item. Therefore, it is concluded that fenoxycarb is not a suitable reference item to be used in such studies and it is recommended to look for another active ingredient which affect the larval development of *O. bicornis* more considerably.

[1] EFSA (2013): EFSA Guidance Document on the risk assessment of plant production products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (published on July 04, 2013, updated on 04 July 2014). EFSA Journal 11(7): 3295.
[2] ICPPR non-*Apis* Workshop (2015): Short Overview of the ICPPR Non-Apis Workshop - Subgroup Higher Tier (Bumble bees and Solitary bees); Limburgerhof, BASF Agrarzentrum, Germany, 19-20 February, 2015.
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[4] OECD (2007): OECD Guidance Document No. 75. Guidance document on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions. - Series of testing and assessment, Number 75. ENV/JM/MONO: 223-27 (2007).
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