Section 2 – Testing effects on honey bee brood

2.1 Detailed brood evaluation under field conditions: advantages and disadvantages

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Abstract

Bee brood studies under semi-field conditions according to OECD GD 75 display a strong variability of the brood termination rates (BTRs) as the key endpoint. Therefore, the ICP-PR Bee Brood Group considered the performance of EPPO 170 field studies using the OECD GD 75 bee brood evaluation as one option to achieve more reliable BTR data. This approach was envisaged already some years ago and used for several years. However, broader data sets supporting the benefit of this combined methodology were still lacking.

The analysis of current field studies performed since 2012 indicate that control BTRs were approximately half the size compared to values observed under semi-field conditions. Moreover, results give a strong indication that the BTR values under field conditions are more reliable and less variabel. Therefore, the combined method is a valuable tool to investigate potential effects of a plant protection product on the bee brood to refine the risk under realistic exposure conditions.

Keywords: Honey bees, detailed brood assessment, brood termination rate, field conditions

Introduction

Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk to honeybee larvae or honeybee brood. According to the not adopted "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA 2013), both, the Oomen bee brood feeding test (Oomen et al. 1992) as well as the OECD Guidance Document 75 (2007) are given as the two higher tier options to refine the risk on honeybee brood if concerns are raised in tier 1.

The evaluation of historical data from semi-field studies according to OECD GD 75 showed a strong variability of the brood termination rates (BTRs) as the key endpoint (Becker *et al.* 2015). Therefore, the performance of EPPO 170 (2010) field studies using the OECD GD 75 bee brood evaluation can be regarded as one option to get more reliable BTR data, which was envisaged previously in 2009 (Becker *et al.* 2009), and followed-up by Giffard & Huart (2015). Moreover, field studies according to EPPO 170 are still considered as the highest tier under EU Regulation 1107/2009/EC. But although in EPPO 170 a broad framework for testing under field conditions is given, no specific and detailed evaluation of the brood development is described. Therefore, EPPO field studies combined with the bee brood evaluation OECD GD 75 could be a useful tool for the honey bee risk assessment.

Material and Methods

Analysed control BTRs from marked eggs derived from assessed brood cycles under field conditions. Five bee brood studies were conducted between 2012 and 2015 in Germany according to EPPO guideline 170 (4) (EPPO 2010) with detailed brood evaluations according to OECD GD 75, *i.e.* marking of single cells containing eggs (= brood area fixing day 0 = BFD 0) and subsequent assessment of larval and pupal development on BFD 5 (±1), 10 (±1), 16 (±1) and 21 (±1) via digital image processing (Pistorius et al. 2012).

The studies covered the assessment of one or two brood cycles during and after the location of the colonies at fields with flowering *Phacelia tanacetifolia* (see Table 1). Control colonies contained sister queens and consisted of two bodies with an appropriate strength. During these studies a total 43 brood cycles (= replicates) were assessed and the corresponding BTRs were obtained (Tab. 1). The studies were mainly carried out under GLP by BASF (Limburgerhof), BioChem (Gerichshain), Ibacon (Rossdorf) and RIFCON (Hirschberg).

The data were compared to the updated findings on control BTRs from 75 semi-field bee brood studies conducted between 2011 and 2015 with BTRs from a total of 299 control colonies (replicates) (Becker *et al.* 2015).

For statistical analysis, the data were natural log-transformed, examined for normal distribution (Shapiro-Wilk test) and homoscedasticity (Bartlett's test), and finally evaluated using the non-parametric Kolmogorov-Smirnov (KS) test as a median test (two-sided, $\alpha = 0.05$). Additionally, under the assumption of equal distribution a Mann-Whitney-U test was performed, too.

Tab. 10 Number of field and semi-field bee brood studies and replicates (colonies) used for data analysis

Type of study	Number of studies [n]	Number of replicates [n]
Field	5	43
Semi-field	75	299

Results

The results show that bee brood studies performed under field conditions display a mean BTR of 14.5% (Table 2), which can be regarded as the natural background level of free flying honeybee colonies. Moreover, this rate is approximately half of the value obtained under semi-field conditions which amounted to a mean of 33.1% Due to the difference and because of the lower variability, BTRs from field studies were statistically significant lower compared to BTRs from semi-field tests ($p<0.001$) (Table 2, Figure 1) in both statistical evaluations. The distribution of the field BTRs to termination ranges shows major differences, too.

Tab. 11 Descriptive statistics of control BTRs obtained under field and semi-field conditions

Type of study	Mean BTR ± SD [%]°	Min BTR [%]	Max BTR [%]	Proportion of repl. with BTRs ≤ 30% [%]°°
Field	14.5 ± 11.7*	1.5	60.3	90.7
Semi-field	33.1 ± 24.4	1.3	100	55.2

° calculated from all replicates (colonies); °° indicator for the reliability of the test method; * statistically significant lower compared to BTRs of the semi-field studies (Kolmogorov-Smirnov test and Mann-Whitney-U-test, $p<0.001$)

A comparison between field and semi-field BTR results illustrates the lower values under field conditions as well as the lower variability (Figure 1). In addition, the results give an indication that under field conditions the number of outliers could be reduced.

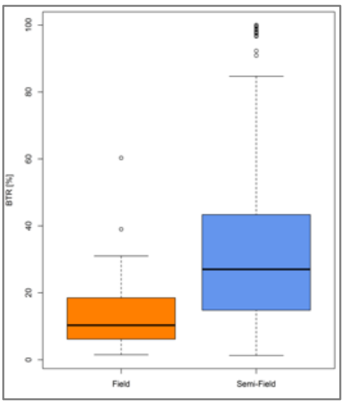

Fig. 1 Box plot of control BTRs from field and semi-field studies (KS-test & U-test, $p<0.001$)

The distribution of the field BTRs to termination ranges shows that a majority of 90.7% of the replicates was ≤ 30%, while under semi-field conditions 55.2% of all replicates reveal BTRs ≤ 30% (Figure 2). Even more the differences of the observed results were pronounced by the proportion of 79% of the replicates display BTRs ≤ 20% under field conditions (Figure 2).

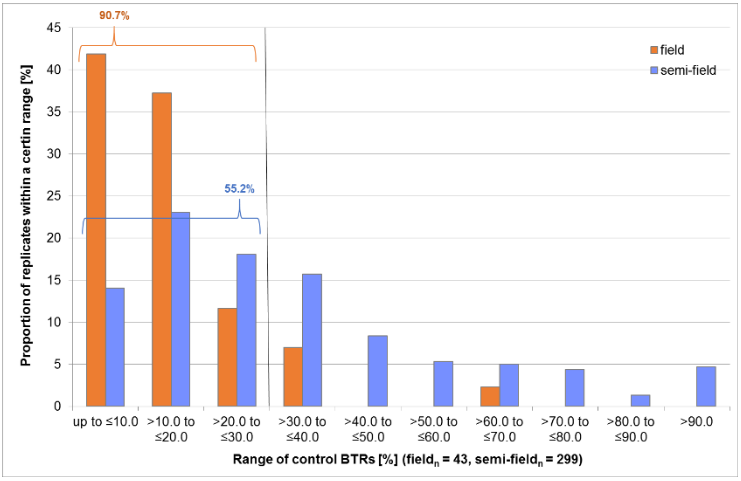

Fig. 2 Distribution of control BTRs of the field and semi-field studies according to size categories

Discussion, conclusion and further steps

Although the number of available field studies is limited compared to the sample size of the semi-field the results give a strong indication that the BTR values under field conditions are lower, more reliable and less variabel.

Based on the presented control BTR data from the field and the findings of OOMEN feeding studies (Lückmann & Schmitzer 2015 & 2017) it can be concluded that the caging situation is an important driver of BTRs. This was already assumed by Becker *et al.* (2015). There, mean BTRs were 21.3% and 14.7% for acute and chronic feeding, respectively (Lückmann & Schmitzer 2015), and thus, were similar compared to the field study results (14.5%). The low mean BTR and the high proportion of replicates displaying BTRs ≤ 30% under field conditions indicate a high reliability of the system which is a clear advantage of this approach. Furthermore, the field conditions display a realistic exposure scenario although it is not a worst-case situation as bees may also forage outside the target crop which is not the case under semi-field conditions. On the other hand, regular managed colonies are used in the field under normal bee keeping practice whereas small sized colonies are employed in the tunnels. Therefore, the combined method is a valuable tool to investigate potential effects of a plant protection product on the bee brood to refine the risk under realistic exposure conditions.

Thus, detailed brood evaluations under field conditions provide a higher reliability to interpret test item results. Consequently, detailed brood assessment under field conditions (EPPO 170) and using free flying colonies (Oomen) can be considered as an useful tool to investigate impact of a PPPs on honey bee brood. A more detailed comparison of the advantages and disadvantages of the methods based on the presentations and publications of the ICPPR Conference 2017 is envisaged as the next step.

It is acknowledged that the presented evaluation of BTRs under field conditions based on a low number of studies comprising detailed brood assessments, especially if compared with the data base from the semi-field. Therefore, it would be important for the future to broader the data base on the one hand and to expand the data base to different countries on the other hand. Moreover, it would be necessary to devlop validity criteria for control BTRs obtained from field studies. This also counts for brood studies according to OECD GD 75, where the discussion was already initiated (Becker et al. 2015)

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