Honeybee brood studies under field conditions: Is there a difference of the brood termination rate compared to semi-field studies?

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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 data requirements under EC 1107/2009 Commission Regulation 284/2013 and the proposed EFSA Bee Guidance Document (EFSA 2014), both the Oomen bee brood feeding test (Oomen *et al.* 1992) as well as the OECD Guidance Document 75 (OECD 2007) (hereafter called OECD GD 75) 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 field studies using the OECD GD 75 bee brood evaluation might be 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). However, broader data sets supporting the benefit of this combined methodology are still lacking.

Thus, the current presentation summarises control BTRs of marked eggs gained under field conditions, compares the findings to the updated findings on control BTRs from semi-field bee brood studies (Becker *et al.* 2015), and discuss the main advantages and disadvantages of both test approaches.

Material & Methods

Analysed control BTRs from marked eggs derived from assessed brood cycles under field conditions. Four 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 39 brood cycles (= replicates) were assessed and the corresponding BTRs were obtained (Table 1). The studies were mainly carried out under GLP by BASF (Limburgerhof), BioChem (Gerichshain) and RIFCON (Hirschberg). The data were compared to the updated findings on control BTRs from 75 semi-field bee brood studies 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, α=0.05). Additionally, equal distribution was assumed and Mann-Whitney-U test was also performed.

Table 1: Number of studies and assessed brood cycles under field conditions

Number of studies [n]	Number of control colonies per study (replicates) [n]	Number of assessed brood cycles		Total number of assessed brood cycles (replicates) [n]
		during the exposure period in the field [n]	After the exposure period at the monitoring site [n]	
2	4 & 7	1	Not assessed	11
2	7 & 7	1	1	28

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). The distribution of the field BTRs to termination ranges shows that a majority of 89.7% of the replicates was ≤ 30% (Figure 2). In contrast under semi-field conditions 55.2% of all replicates reveal BTRs ≤ 30%.

Table 2: Descriptive statistics of control BTRs obtained under field and semi-field conditions

Type of study	No. of studies [n]	No. of repl. [n]	Mean ± SD [%]°	Min. [%]	Max. [%]	Prop. of repl. with BTRs ≤ 30% [%]°°
Field studies	4	39	14.5 ± 12.1*	1.5	60.3	89.7
Semi-field studies	75	299	33.1 ± 24.4	1.3	100	55.2

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

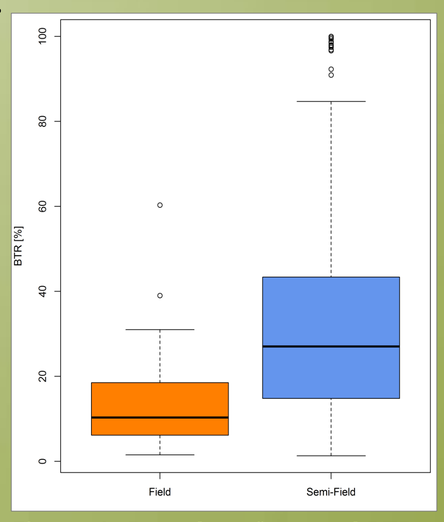

Figure 1: Box plot of control BTRs (KS-test & U-test, $p<0.001$)

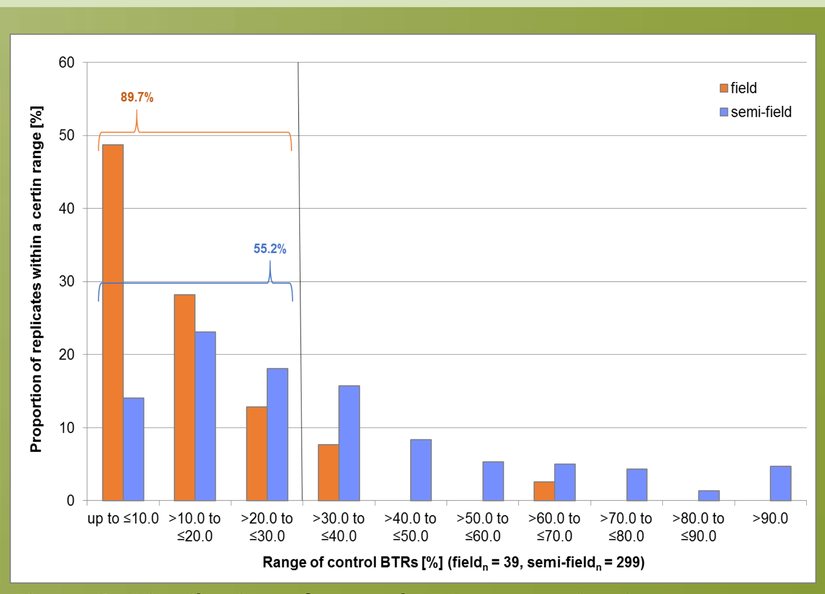

Figure 2: Distribution of control BTRs to termination ranges

Discussion

The findings indicate that the artificial tunnel conditions (`caging effect') are one of the major factors influencing the brood termination in honeybee colonies which was already assumed by Becker *et al*. (2015) when comparing the semi-field BTRs to those obtained at Oomen feeding studies using free flying honeybee colonies. 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. 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.

Conclusions

Brood termination rates obtained under field conditions are presented and compared to values obtained under semi-field conditions. Overall the results show that detailed brood investigation under field conditions according to OECD GD 75 generally displays lower and less variable BTRs and exhibit a higher reliability of the test system compared to semi-field bee brood studies. 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.

Literature

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