3.3 ICP-PR Bee Brood Working Group – Variability of brood termination rates in reference to validity criteria and limited effectiveness of method improvement in honeybee semi-field studies (OECD GD 75)

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Abstract

OECD Guidance Document 75 (2007) outlines a test method to assess effects of plant protection products (PPPs) on honeybee brood under semi-field conditions. The assessment of bee brood development is conducted by mapping cells containing eggs and following their development until emergence. Evaluated parameters are: brood termination rate (BTR), brood compensation index (CI) and brood index (BI). Due to high variability of BTRs within treatments and high control mortality in a number of studies no definite conclusions regarding effects on brood were possible in the past and studies needed to be repeated (Pistorius *et al.* 2012). To address this variance, effort was taken by ICP-PR and AG Bienenschutz to improve the method by further analyzing current and historical data considering possible influencing factors (Pistorius *et al.* 2012, Becker *et al.* 2015) to give recommendations for future testing. The main findings were that reliability of the test method was questionable and that further method improvement and data evaluation was required. Therefore in this paper data evaluation of studies conducted between 2014 and 2017 is carried out and potential key parameters influencing outcome of studies are given.

To evaluate the improvement of the OECD 75 test method following the recommendations from 2015, a data analysis of 86 studies conducted in Germany, France, Spain and US was performed. The mean BTR value in the control group was 30.2% for studies conducted in Germany (mean of 61 studies), 19.4% in France (mean of 3 studies), 41.8% in Spain (mean of 5 studies) and 50.6% in US (mean of 17 studies). Results from Spain and US displayed higher BTRs in control compared to data from Germany. Evaluation of BTRs for Germany displayed only a slight improvement (historical value of 32.9%).

Analysis of data shows a limitation of options to improve the method as no main driver for high variability of BTRs in the control group was found. The cause for low precision may be multifactorial and driven by "caging effect". There are alternative test methods available to observe bee brood development, without confinement in the tunnels, under field conditions (Oomen *et al.* (1992), OECD GD 75 field test design). Therefore it is necessary to investigate differences between these open field methods and semi-field testing with regard to routes of exposure, residues in brood and brood mortality, to choose the most reliable and adequate testing method assessing potential effects of PPP on honeybee brood development.

Keywords: OECD GD 75, brood termination rate, semi-field studies

Introduction

OECD GD 75 (2007) was developed to detect adverse effects of plant protection products (PPP) on honeybee brood under worst-case semi-field conditions, which is necessary especially for products affecting insect development like insect growth regulators (IGR). The endpoints measured according to OECD GD 75 are very closely related to the mode of action and the properties of the PPP. Unfortunately, the results display a high variability limiting the detectability of small effects in a reliable way. To address this variance, effort was taken by ICP-PR and AG Bienenschutz to improve the method by further analyzing current and historical data considering possible influencing factors (Pistorius *et al*. 2012, Becker *et al.* 2015) to give recommendations for future testing. The main findings were that reliability of the test method was questionable and that further method improvement and data evaluation was required. Therefore in this paper data evaluation of studies conducted between 2014 and 2017 is carried out and potential key parameters influencing outcome of studies are given.

Material and Methods

To obtain results for data evaluation contract research organizations and companies producing plant protection products were asked to submit data on control and reference item from semi-field bee brood studies conducted according to OECD GD 75 and Pistorius *et al.* (2012).

For each colony the following parameters were requested:

- Brood termination rate (BTR)
- Day of the year at BFD0 (brood fixing day)
- Colony strength
- Number of cells with brood, pollen, nectar/honey at BFD0
- Number of cells marked at BFD0
- Number of cells with pollen, nectar/honey on marked and adjacent combs at BFD0
- Application rate of the reference item (a.i.: fenoxycarb)
- Number of days in the tunnel before and after application
- Weather conditions during study: min, max and mean air temperature, mean air humidity, rainfall

In total data from 86 studies conducted under GLP in Germany, France, Spain and US by BioChem agrar, Eurofins Agroscience, ibacon, RIFCON, BASF SE, BayerCropscience, Dow AgroSciences, E. I. duPont de Nemours and Company, FMC and Syngenta were provided. The overview about number of studies and replicates from each country for control and reference item is given in Tab.1.

Table 1 Number of semi-field brood studies provided for the evaluation

Country*	Number of studies [n]	Number of replicates** (tunnels) [n]	
		Control	Reference item
Germany	61	243	212
France	3	12	12
Spain	5	19	15
US	17	68	48

*number of studies with mean BTR>50% in control: Germany: 14, Spain: 3, US: 7

**requested parameters were not available for all replicates

All studies were conducted between 2014 and 2017 with exception of 3 studies conducted in 2009 and 2010 which were not part of data evaluation presented in Pistorius *et al.* 2012 and Becker *et al.* 2015. From all requested parameters only BTR values and the brood fixing date (=BFD0, initial assessment of brood development) were available for all control and reference item replicates. Due to incompleteness of provided data, only studies conducted in Germany were taken into consideration. From 61 studies done in Germany, 4 of them were conducted in winter oil seed rape and 57 in *Phacelia tanacetifolia*. From all requested parameters four were identified as potentially influencing brood development, i.e. colony strength, day of the year at BFD0, total number of cells containing pollen per colony at BFD0 and weather condition (max. air temperature and sum of precipitation during exposure). Potential influence on level of control BTR was evaluated for: day of the year at BFD0 (n=243 colonies), colony strength at BFD0 (n=182), number of cells containing pollen per colony at BFD0 (n=74), max. air temperature (n=180) and sum of precipitation during the exposure phase (n=92).

Results of semi-field brood studies conducted in Germany

Brood termination rate

A summary of the current data evaluation on BTRs from studies conducted in Germany is given in Tab. 2. Additionally, historical data from two evaluations done in the past (Pistorius *et al.* 2012, Becker *et al.* 2015) are presented in Tab. 2.

Table 2 Summary of current data evaluation on BTRs including historical data

Parameter	Brood termination rate (BTR) [%] <2011* Control n=63	Reference item n=54	2011-2014** Control n=208°(n=239)	Reference item n=192° (n=207)	2014-2017*** Control (n=243)	Reference item (n=212)
Median	25.9	83.4	23.4 (26.5)	77.4 (75.0)	21.4	86.3
Mean	34.7	76.8	29.2 (32.9)	70.7 (70.4)	30.2	72.0
SD	24.8	24.2	21.6 (24.4)	27.4 (27.3)	26.8	30.4
Min	4.9	20.9	2.0 (2.0)	2.6 (2.6)	0.9	5.8
Max	100	100	100 (100)	100 (100)	100	100

n=number of replicates (colonies), *Pistorius *et al.* 2012, **Becker *et al.* 2015, ° 8 studies excluded, ***current evaluation

Variability of control BTRs

In the OECD GD 75 there is no validity criterion for brood (eggs) mortality proposed nor requested.

Becker *et al.* (2015) assumed that reliability of the test system is indicated when BTRs are on a low level. Similar to the evaluation done by Becker *et al.* (2015), the number and distribution of control replicates with BTRs ≤30% and ≤40% were evaluated and are given in Tab. 3 and Fig. 1. In 55.6% of the control replicates (studies <2011 and 2011-2014) the BTRs were ≤30%. Current results show that proportion of replicates with BTRs ≤30% increased to 65.0%. Number of replicates with BTRs ≤40% increased from 70.7% (2011-2014) to 77.0% in the current evaluation. Fig. 1 shows that the number of replicates with BTRs ≤10% increased and was obtained for 21% of replicates, whereas the number of replicates with BTRs ≥80% and ≥90% increased to 2.1% and 7.0%, respectively.

Table 3 Proportion of replicates with low and high BTRs including historical values

Proportion of replicates with BTRs	% of replicates <2011* Control n=63	2011-2014** Control n=208°(n=239)	2014-2017*** Control (n=243)
≤30%	55.6	61.5 (55.6)	65.0
≤40%	68.3	76.9 (70.7)	77.0

n=number of replicates (colonies), *Pistorius *et al.* 2012, **Becker *et al.* 2014, ° 8 studies excluded, ***current evaluation

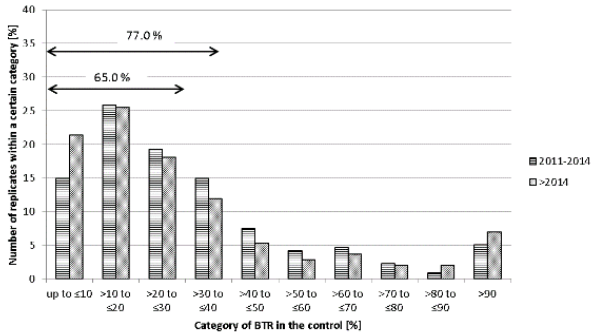

Figure 1: Histogram of control BTRs

Colony strength at BFD0 in control replicates and its influence on brood development

Colony strength (number of adult bees per colony at BFD0) was compared with BTRs evaluated on the last assessment of brood development. The results are given in Fig. 2.

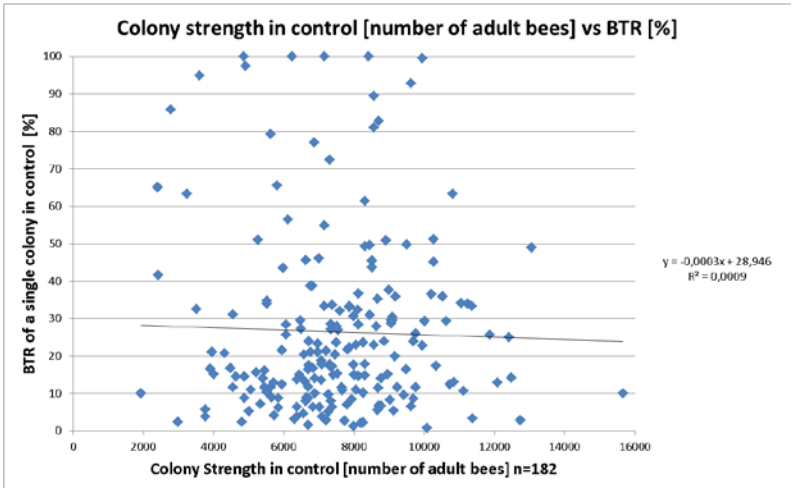

Figure 2 Colony strength in control [number of adult bees] vs. BTR [%]

Colonies with 5000 to 10000 adult bees and higher, display a slightly higher probability to obtain lower control BTRs at the end of the study, while in the historical data (Becker *et al.* 2015) colonies with 6,000 to 8,000 bees displayed a higher probability to obtain BTRs ≤ 30% (chi^2-test, p=0.019).

Start of the study in the season (day of BFD0)

Date of BFD0 was provided for all control replicates. Evaluation was limited to studies conducted in *Phacelia tanacetifolia*.

No significant correlation was found (y=0.125x + 6.1943, R^2=0.0111). It is assumed that for studies starting before end of July there is a slightly higher probability to obtain control BTRs≤40% than for those starting after 1st of August.

Total number of cells containing pollen per colony at BFD0

Total number of cells containing pollen per colony in control replicates (n=74) at BFD0 was compared with BTRs evaluated on the last assessment of brood development. No significant correlation was found: R^2=0.0799 (y=-0.0026x + 35.502).

Weather conditions during exposure phase

For n=180 control replicates maximum air temperature was available and compared with BTRs evaluated on the last assessment of brood development. No significant correlation was found: y= 0.5205x + 8.1528, R^2= 0.0212.

For n=92 control replicates sum of precipitation during exposure phase was provided. These numbers were compared with BTRs at last assessment. No significant correlation was found: y= - 0.1855x + 28.459, R^2= 0.0526.

Results of semi-field brood studies conducted outside Germany

Since the OECD GD 75 was originally developed and designed for central EU, *Phacelia tanacetifolia*, Mini-Plus hives and *Apis mellifera carnica*, any implementation and extrapolation of reference data to other climatic zones, other crops (e.g. buckwheat), other hive sizes and bee species should be done very carefully and with expert judgement only.

Discussion and conclusion

The main results of the historical data (Becker *et al.,* 2015) were confirmed by current evaluation regarding studies conducted in Germany: no distinct improvement of BTRs was found and a high variability within the respective studies remains, with still a high proportion of replicates with control BTR ≥30%. The main driver is still not identified, but most likely driven by the „caging effect". It still remains unverified (was not considered in any of the data evaluations) how the preparation of the hives before initiation of the studies influence their outcome.

Discussion within the ICP-PR working brood group is needed on other existing test methods assessing bee brood development under field conditions (Oomen *et al.* (1992), OECD GD 75 field test design). In the colony feeding test design according to Oomen *et al.* (1992) a different route of exposure and dilution of the residues may occur since the exposure is only via sugar solution and bees are free-flying, foraging on surrounding crops. In the OECD GD 75 field test design bees may forage on surrounding crops (realistic exposure in agriculture), but in comparison to the semi-field test design the worst-case exposure is not given. It is necessary to investigate differences between these methods and semi-field testing in regards to routes of exposure, residues in brood and brood mortality to choose the most reliable and adequate testing method assessing potential effects of PPP on brood development. In addition, interpretation of data and their use for the evaluation of the risk to honeybees should be reconsidered: in case of high BTRs, the BTRs obtained in the control may be put in relation to BTRs in reference item treatment. Other possibility could be prolongation the study over the second brood cycle in case of a strong „caging effect". Factors other than brood termination rate may also be more reliable and valuable endpoints when determining effects on brood development, for example the compensation index.

The test method (OECD GD 75) is currently the only available possibility to investigate potential effects of PPP on brood development under semi-field, conditions (realistic worst case) when both, exposure to treated nectar and pollen are given.

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