Effectiveness of method improvements to reduce variability of brood termination rate in honey bee brood studies under semi-field conditions

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Abstract

Quantitative assessments of adverse effects of plant protection products on honey bee brood (*Apis mellifera* L.) may be carried out according to the methods given by the OECD Guidance Document No. 75 (2007). In recent years a number of studies displayed a strong variability in brood termination rates, a key endpoint. Due to these variances no definite conclusions regarding potential brood effects were possible, and the studies needed to be repeated. Due to this, attempts to improve the methodology were initiated by the Working Group 'Honey bee brood' of the German AG Bienenschutz. In 2011, honey bee brood studies adapted to these identified possible improvements resulted in better results compared to historical data. Based on the analysed results, the working group recommends to improve the method by using bigger colonies with more brood, using 4 instead of 3 replicates for better interpretation of data, starting the study early in the season, avoiding major modifications of the colonies shortly before application and using larger tunnels with effective crop areas preferably > 80 m^2. To carry out quicker brood cell assessments to reduce stress for the colonies, it is recommended to use digital brood assessment, which allows marking a higher number of cells (e.g. 200 to 400 cells).

Introduction

One recently used methodology to investigate the honey bee brood development under realistic exposure conditions are semi-field studies according to Schur *et al.* (2003) superseded by the OECD Guidance Document No. 75. In the course of the last few years it became obvious that the brood termination rate (= mortality of bee brood in selected cells on combs) was subject to a certain degree of variation, e.g. resulting in replicates with increased rates up to 100% in the control and reduced rates in the reference item group down to 21%. Additionally, a high variation between replicates within a respective treatment group occurred sometimes. The variability which was distinctly more present under semi-field conditions compared to a field method (Oomen *et al*. 1992) complicates the interpretation of results regarding potential brood effects of the test items with the outcome that some studies were regarded as invalid. The time between BFD 0 (Brood area Fixing Day) and BFD 5 turned out to be the most critical for such variations. To improve the current methodology, the Working Group 'Honey bee brood' of the AG Bienenschutz discussed some aspects of the method, e.g. timing of the experiment, crop area, size and composition of bee colonies, digital comb vs. acetate sheet assessment of brood cells in spring 2011 (Pistorius unpubl.; Becker & Lückmann 2011). The effectiveness of some of these factors were investigated in the subsequent season 2011. First improvements in the experimental procedure were identified which are presented in this paper and which may result in a proposal for an addendum to the existing OECD Guidance Document.

Material and methods

At the meeting in spring the 2011 the following measures for improvement were proposed, summarized in the table below(Table 1).

Tab. 1 Summary of measures to be improved in 2011 and given by the OECD GD 75

Parameter	According to OECD GD 75	Proposed improvement
Colony size	Small test colony (e.g. Mini Plus, nuclei), ~ 3,000 brood cells (≙ 750 cm^2) with brood in all stages, 1 food comb with honey and pollen, ~ 800 g (= ~ 6,000) worker bees	Colonies (nuclei) with 10 frames, 3-5 brood combs, high proportion of capped cells
Crop area	≥ 40 m^2 per tunnel	≥ 80 m^2 per tunnel
Reference item	Insegar, application rate ≥ 600 g/ha ≙ 150 g fenoxycarb/ha (single rate)	single or double rate
Timing	not specified	early start in the season
Irrigation	not specified	if the field is dry
Brood assessment	acetate sheet method; ≥ 100 cells/colony	digital photo method; ≥ 200 cells/colony

For the analysis of potential factors influencing the variability of bee brood studies the following data sets were used:

- period 2002 and 2010: 21 studies with 63 replicates in the control and 54 replicates in the reference item. The data analysis was presented by Becker & Lückmann (2011) and are called 'historical data' in the following.
- 2011: 13 studies (total of 50 replicates) for the control data and 12 studies (total of 43 replicates) for the reference item fenoxycarb (1 study was carried out with dimethoate and was therefore not considered); since in some studies the number of replicates in the toxic reference was lower than in the control, the total number of replicates was different in both groups.

The following endpoints were analysed for its relevance on brood termination rates (BTR) in the control:

- time of the year, expressed as 'day of the year'
- effective crop area
- colony strength

For the reference item the following endpoints were analysed for its influence on the brood termination:

- larval/pupal mortality

Only studies carried out on *Phacelia tanacetifolia* were considered.

Results

a) Influence of study initiation on brood termination rate in the control

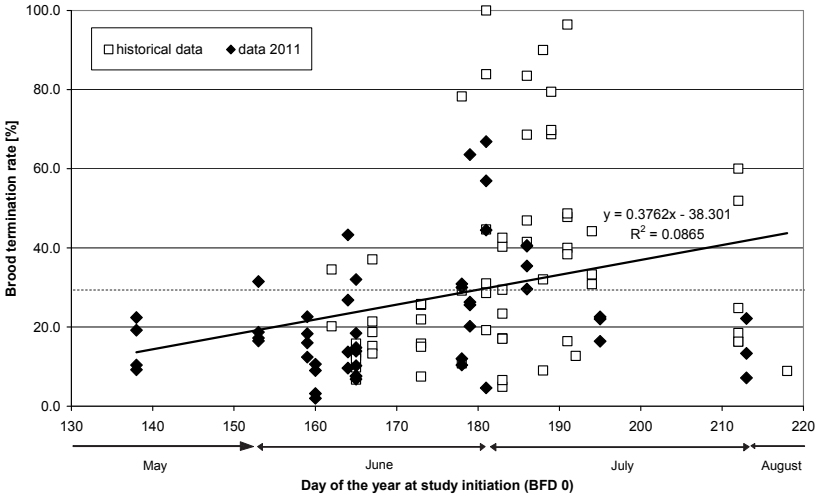

Fig. 1 Influence of study initiation (BFD0) on brood termination rate in the control

The analysis indicate that studies which were initiated before end of June (~ day 181) displayed an increased probability to achieve BTRs ≤ 30% in the control.

b) Influence of effective crop area on brood termination rate in the control

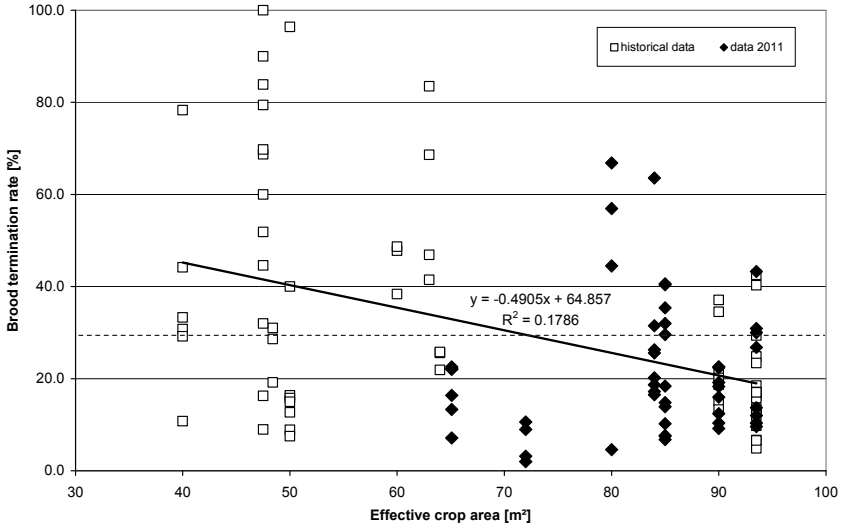

Fig. 2 Influence of effective crop area on brood termination rate in the control

The results show that studies with increased crop areas resulted in higher probabilities to obtain BTRs ≤ 30% in the control.

c) Influence of a combination of study initiation and effective crop area on brood termination rate in the control

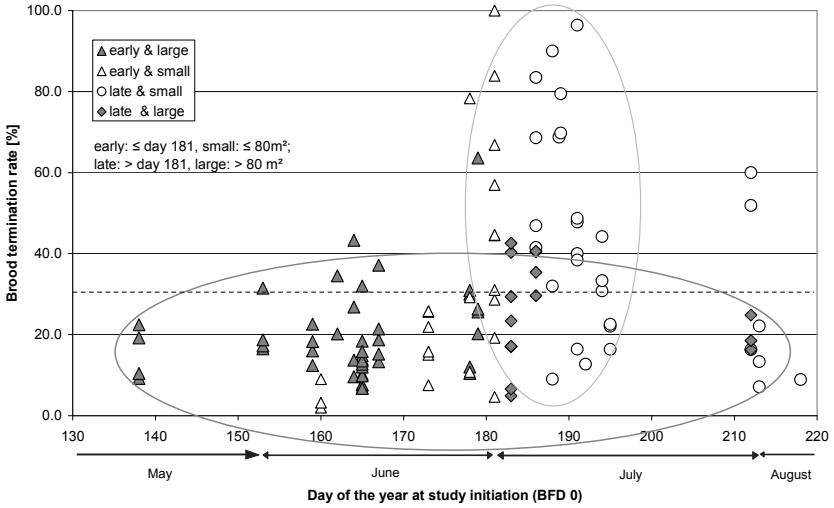

Fig. 3 Influence of study initiation and effective crop area on brood termination rate in the control

A combined analysis of the influence of study initiation and crop area shows that studies which were performed until end of June and/or were performed in tunnels with effective crop areas > 80 m² display higher probabilities to obtain BTRs ≤30%. In contrast, studies which were performed in tunnels with effective crop areas ≤80 m² and carried out after end of June display higher probabilities to result in BTRs > 30%.

d) Influence of colony strength on brood termination rate in the control

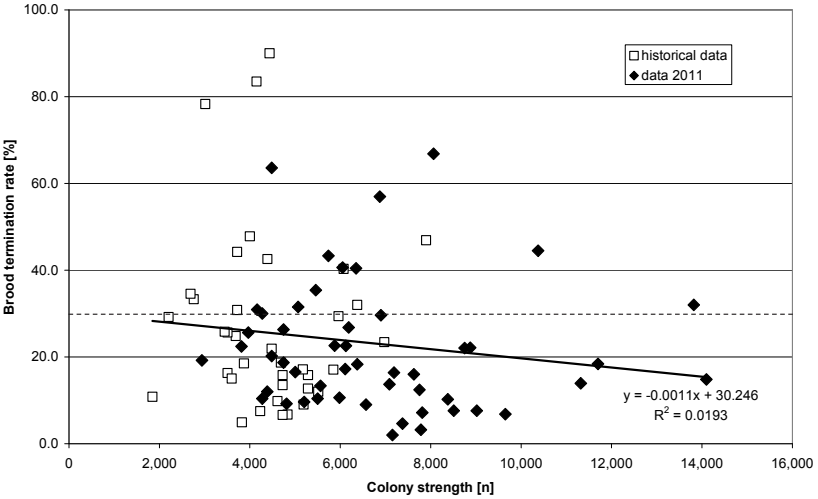

Fig. 4 Influence of colony strength on brood termination rate in the control

The analysis shows that studies which were performed with colony strengths higher than approximately 7,000 bees display higher probabilities to achieve BTRs ≤30%.

e) Influence of decreased brood termination rate on pupal mortality in the toxic reference

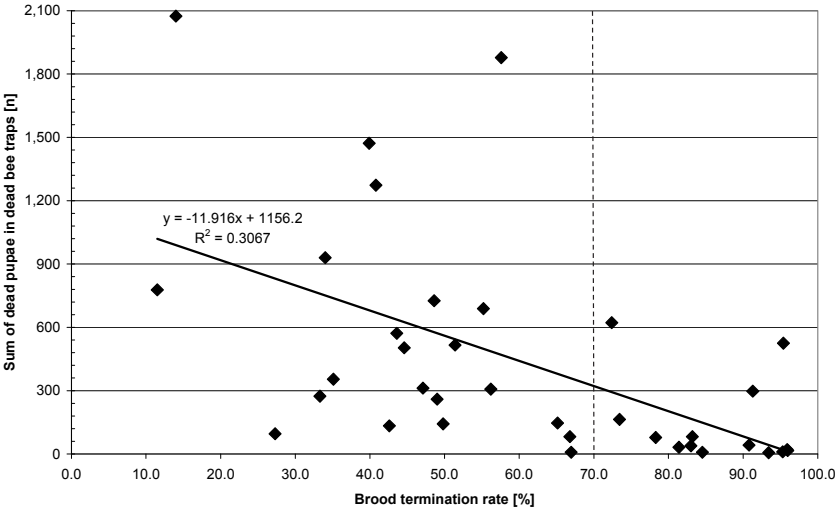

Fig. 5 Influence of decreased brood termination rate on pupal mortality in the toxic reference

The analysis shows that replicates with low BTRs, e.g. < 70% often display an increased pupal mortality, indicating that a sufficient exposure of the honey bees had took place and thus the suitability of the test system to detect potential effects on the bee brood. Only one replicate out of 22 (95.5%) replicates with BTR ≤70% displayed no increased pupal mortality.

Discussion and conclusion

The results show that the suggested improvements led to a reduction of BTRs and variability in the control group of honey bee brood studies in 2011, when compared to the historical data (see Table 2). Nevertheless the proposed measures cannot be a 100% guarantee to obtain always studies with BTRs ≤30%. Even using the proposed improvements (study initiated before end of June and/or use of large effective crop areas) studies in 2011 demonstrated that BTRs might be distinctly higher than 30% due to unknown reasons. For this reason it is important to analyse the importance of further factors in the future.

Tab. 2 Summary and comparison of descriptive statistics of historical and current bee brood studies

	Brood termination rates [%]			
	Historical data		**Data 2011**	
	Control (n=63)	**Toxic reference (n=54)**	**Control (n=50)**	**Toxic reference (n=43)**
Mean	34.7	76.8	21.7	63.7
SD	24.8	24.2	14.8	21.1
Median	25.9	83.4	18.4	65.1
Minimum	4.9	20.9	2.0	11.5
Maximum	100	100	66.8	100
Proportion of replicates ≤30% in the control and > 70% in the toxic reference	55.6	70.4	78.0	41.4*

*95.5% of these replicates display a pupal total mortality > 80 pupae during the entire study period

Based on the experiences and results obtained by the improved honey bee brood studies in 2011, the Working Group 'Honey bee brood' of the AG Bienenschutz recommends:

- to use bigger colonies with 3 to 4 brood combs, containing a high number of capped cells,
- to avoid major modifications of the colonies shortly before application,
- to use 4 instead of 3 replicates for better interpretation of data,
- to start the study early in the season, if possible,
- to use large tunnels, which provide effective crop area > 60 m^2, preferably > 80 m^2,
- to water the crop if dry conditions reduce nectar flow,
- to evaluated termination rate and pupal mortality in the toxic reference item.

Although the digital brood cell assessment has several advantages (e.g. quicker assessments, reduced stress for colonies) there was no correlation between BTR and the use of acetate sheets vs. digital brood cell assessment (Jeker et al. 2011 and 2012, Wang & Classen 2011) and the observation of higher numbers of marked cells. Nevertheless, the Working Group recommends:

- to use digital brood cell assessment,
- to observe 200 to 400 cells.

To verify the improvements and to identify possible additional ones, the work will be continued in 2012. At the end, the authors hope to give recommendations for an improvement of the OECD GD 75 intended to be developed until end of 2012.

Whereas the OECD GD 75 is used as a guidance for honey bee brood studies in the EU the recommendations are based on data from studies carried out in Central Europe, i.e. Germany and Switzerland. Therefore it will be necessary to include experience and recommendations also from others parts of Europe, e.g. Southern Europe.

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References

1. Becker R & Lückmann J (2011): Variability of brood termination rate in honey bee brood studies under semi-field conditions. 58. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung e.V., Berlin-Dahlem 29. - 31.3.2011.
2. Jeker L, Meschberger T, Candolfi M & Magyar J P (2011): Digital image analysis as a tool to improve the assessment and evaluation of brood development in higher tier honey bee studies. Workshop of AG Bienenschutz on Improvement of Bee brood studies, Julius Kuehn-Institut (JKI) February 23th, 2011.
3. Jeker L, Schmid L, Meschberger T, Candolfi M, Pudenz S, Magyar J P (2012): Computer-assisted digital image analysis and evaluation of brood development in honey bee combs. Journal of Apicultural Research 51(1), 63-73.
4. OECD Guidance Document No. 75 (2007): Guidance document on the honey bee (Apis mellifera L.) brood test under semi-field conditions. Series of testing and assessment, Number 75, 3-27.
5. Oomen PA, De Ruijter A & Van der Steen J (1992): Method for honey bee brood feeding tests with insect growth-regulating insecticides. OEPP/EPPO Bulletin 22, 613 – 616.
6. Pistorius J. unpubl.: AG Bienenschutz: Protocol of the meeting at Julius Kühn Institute (JKI) on February 23th, 2011.
7. Schur A, Tornier I, Brasse D, Mühlen W, von der Ohe W, Wallner K & Wehling M (2003): Honey bee brood ring-test in 2002: method for the assessment of side-effects of plant protection products on the honey bee brood under semi-field conditions. Bulletin of Insectology 56 (1), 1 – 7.
8. Wang M & Claßen C (2011): Automated evaluation of honey bee brood trials using digital image processing according to OECD 75, Oomen (1992) and beyond. Poster presentation at SETAC North America, November 2011, Boston.