Poster

Section 2 - Risk Assessment/ Microbials

Brood termination rate in honey bees in two consecutive brood cycles: a comparison

Lückmann, Johannes[1]*; Bluhm, Wally[1]; Kimmel, Stefan[2]; Steeger, Thomas[3]; Wilkins, Selwyn[4]

[1]RIFCON GmbH, Hirschberg, Germany

[2]Corteva Agriscience, Munich, Germany

[3]US Environmental Protection Agency, Washington, USA

[4]Fera Science Ltd., York, UK

*email: johannes.lueckmann@rifcon.de

Abstract

Semi-field studies of honey bee (*Apis mellifera* L.) colonies provide an important mean of assessing the effect of chemical exposure on brood development. Brood termination rate (BTR) is a common metric for evaluating effects of exposure; however, the index can be variable thereby limiting the extent to which studies can detect treatment-related effects. This study evaluated whether BTR in successive brood cycles differs between the enclosure phase vs monitoring phase of semi-field studies. The data indicate that for controls, differences were not statistically different; however, for colonies exposed to the reference toxicant fenoxycarb, BTR was significantly ($p < 0.05$) higher during the enclosure phase compared to the monitoring phase.

Keywords: honey bee, brood termination rate, BTR, consecutive brood cycles

Introduction

The potential impact of pesticides on developing honey bee (*Apis mellifera* L.) eggs, larvae and pupae (*i.e.*, brood) is often investigated under semi-field, worst-case exposure conditions, according to OECD GD 75 (OECD 2007), with the brood termination rate (BTR) as one of the key measurement endpoints to be considered. Historical data from such semi-field studies, where brood cells with eggs are marked out and the 7-day exposure period takes place under tunnel conditions, show high variability in the BTRs within the untreated control groups (Pistorius *et al.* 2012, Becker *et al*. 2015, Szczesniak *et al.* 2018). In contrast, control BTRs recorded in similar studies run under field conditions with free-flying honey bees are substantially lower and less variable (Lückmann & Tänzler 2020).

The current analysis by the International Commission for Plant Pollinator Relationships (ICP-PR) Bee Brood Working Group investigated the magnitude and variability of BTRs for a negative control and a reference chemical (*i.e.*, the insect growth regulator [IGR] fenoxycarb) over two subsequent brood cycles. The first started under semi-field conditions (*i.e.*, confinement of colonies in the tunnels), while the second was initiated under full-field conditions after completion of the first brood cycle when colonies have been removed from the tunnels to a monitoring site. In addition, the results obtained for the reference chemical fenoxycarb provide insight into the duration of effects caused by this chemical, an insect growth regulator (IGR) known to affect larval development. The results are discussed regarding the interpretation of BTRs gathered from such bee studies.

Poster

Material and methods

For the evaluation, data from ten semi-field bee brood studies comprising a total of 44 control and 40 reference item nucleus colonies (application rate: 300 g fenoxycarb/ha, one study with 480 g fenoxycarb/ha) were available. The BTRs of marked eggs (BTR_{eggs}) at the end of the 1st (~BFD22) and 2nd brood cycle (~ BFD44) were analysed. The studies were conducted according to OECD GD 75 (OECD 2007) and current improvements (Pistorius *et al*., 2012, Becker *et al.* 2015) under Good Laboratory Practice (GLP) standards in Germany and Switzerland. A bee-attractive crop (*i.e*., *Phacelia tanacetifolia*) was used during the tunnel phase with an area between 82 m² and 126.5 m². The studies were performed between 2015 and 2020.

The statistical analysis was performed for a comparison of the BTRs of the 1st vs. 2nd brood cycle for each treatment group (two-sided) and for a comparison of the 1st and 2nd brood cycle for the control vs. fenoxycarb (one-sided greater). Normal distribution was tested with Shapiro-Wilk test, followed by Wilcoxon rank sum test with continuity correction for not normally distributed data, $\alpha = 0.05$. Program: R, version 4.0.5 (2021).

Results

The results (BTRS) are summarised in Table 1 and graphically illustrated in Figure 1.

Control:

In the 1st brood cycle, the mean BTR of 31.7% and proportion of colonies with BTRs ≤ 30% / ≤ 40% (*i.e*., 61% / 75%) were similar to historical control data as described by Becker *et al*. (2015) and Szczesniak *et al*. (2018) with a mean BTR of about 30% and proportions of about 61.5% to 65% and 77%, respectively.

In the 2nd brood cycle, the mean BTR of 22.1% was lower compared to the 1st brood cycle, but was not statistically significantly different. The mean BTR and proportion of colonies with BTRs ≤ 30% / ≤ 40% (*i.e*., 75% / 86%) were comparable to levels observed in free flying colonies as described by Lückmann & Tänzler (2020) with a mean BTR between 16% to 20% and proportions of about 80% to 90% and 87% to 90%, respectively. Finally, 86% of the colonies displayed a decrease in the BTR or the BTR remained at an already low level.

Reference item:

In the 1st brood cycle, the mean BTR of 71.4% was comparable with levele observed for historical reference item data as described by Becker *et a*l. (2015) (*i.e.,* 71%). Also, the proportion of colonies with BTRs $\geq$ 70% was comparable to historical data (*i.e*., 60%) compared to 58% (ICPPR unpubl.). The studies indicate that 13% of the colonies displayed BTRs $\leq$ 30% and 20% of the colonies had BTRs $\leq$ 40%.

In the 2nd brood cycle, the mean BTR of 26.4% and the proportion of colonies with BTRs $\leq$ 30% / $\leq$ 40% were similar to the control level. Almost no colonies with BTRs $\geq$ 70% were observed. The studies indicate that 83% of the colonies displayed a decrease in BTR and 55% of the colonies reached the control level of the 2nd brood cycle.

Figure 1 depicts box plots of control and reference colony BTRs for both brood cycles. The statistical analysis displayed a significant difference between the 1st and 2nd brood cycle ($p < 0.001$) within reference item group and between the control vs reference item for the 1st brood cycle ($p < 0.001$) but not for the 2nd cycle.

Poster

Table 1 *Descriptive statistics of brood termination rate for honey bee eggs (BTR_{eggs}) in the control and reference item (fenoxycarb) group at two subsequent brood cycles.*

BTR_{eggs}°	Control		Reference item	
	1st brood cycle	2nd brood cycle	1st brood cycle	2nd brood cycle
Minimum [%]	3.7	3.3	32.8	3.4
Mean ± SD [%]	31.7 ± 28.4	22.1 ± 18.1	71.4 ± 29.6*,**	26.4 ± 19.2
Maximum [%]	100	46.0	100	90.1
Proportions of replicates with BTR_{eggs} ≤ 30% / ≤ 40%	61 / 75	75 / 86	13 / 20	65 / 80
Proportions of repl. with BTR_{eggs} ≥ 70%	11	5	60	3

*°calculated from all replicates; * = statistically significant different from the control (1st brood cycle), p <0.001; ** = statistically significant different between 1st and 2nd brood cycle (reference), p <0.001*

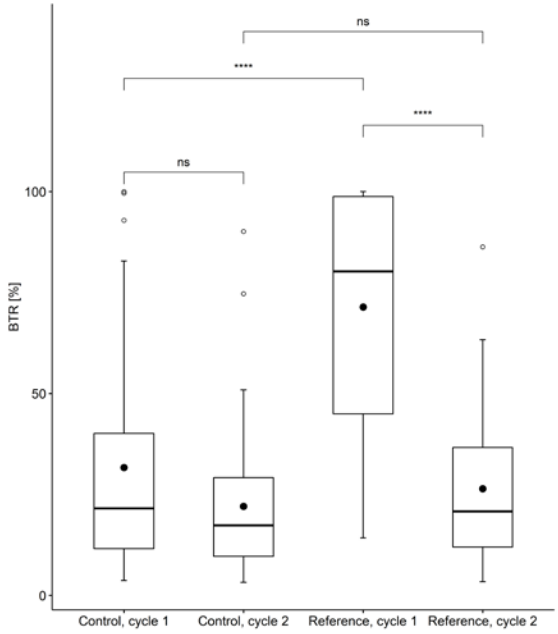

Figure 1 *Box plot of brood termination rate (BTR) for marked eggs in the control and reference item (fenoxycarb) group at two subsequent brood cycles (filled dots = mean, solid line = median, unfilled dots = outliers, ns = not statistically significant different, **** = statistically significant different with p <0.001)*

Discussion and Conclusion

The findings indicate that:

- the caging effect dissipates when honey bee colonies are removed from the tunnels

- the effects on the bee brood in the fenoxycarb group generally lasts for one brood cycle, dissipating in the subsequent cycle

- further investigations are needed (*e.g.*, on setups with chemicals that have proven long-lasting effects or effects persisting beyond the 1st brood cycle; reversed setup with 1st brood cycle started outside the tunnels followed by the 2nd brood cycle with a brood fixing evaluation under semi-field conditions)

- based on the available data, it is sufficient to analyse the detailed brood development during one brood cycle

- however, to broaden the database, more companies are asked to contribute their data sets for further evaluation.

Acknowledgements

Many thanks to all the contract labs (BioChem agrar, Eurofins, IES and RIFCON) and companies (BASF SE, Corteva Agriscience and Trifolio) for providing their data. Thanks also belong to Dr. B. BAUER (RIFCON) who performed the statistical analysis.

References

BECKER, R., LÜCKMANN, J., PISTORIUS, J., 2015: Effectiveness of method improvements of OECD GD 75 – Evaluation of the ICP-PR Bee Brood Working Group. Julius-Kühn-Archiv **450**, 83-92.

LÜCKMANN, J., TÄNZLER, V., 2020: Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: is there a difference of the brood termination rate? Julius-Kühn-Archiv **465**, 91-95.

OECD (2007): 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(2007)22, 27 p.

PISTORIUS, J., BECKER, R., LÜCKMANN, J., SCHUR, A., BARTH, M., JEKER. L., SCHMITZER. S. VON DER OHE. W., 2012: Effectiveness of method improvements to reduce variability of brood termination rate in honey bee brood studies under semi-field conditions. Julius-Kühn-Archiv **437**: 115-120.

SZCZESNIAK, B., PILLING, E., BOCKSCH, S., BECKER, R., LÜCKMANN, J., 2018: 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). Julius-Kühn-Archiv **462**: 111-115.