Evaluation and Improvements of the Oomen *et al.* Bee Brood Test

S. Schmitzer[1)], J. Lückmann[2)], on behalf of the German AG Bienenschutz

[1)] IBACON GmbH, Arheilger Weg 17, D-64380 Rossdorf, Germany, E-mail: stephan.schmitzer@ibacon.com
[2)] RIFCON GmbH, Goldbeckstraße 13, D-69493 Hirschberg, Germany, E-mail: johannes.lueckmann@rifcon.de

Introduction

Recent developments in the risk assessment of plant protection products (PPP) on bees (EU Regulation 1107/2009, EFSA 2013) promote the evaluation of potential effects on honeybee brood (*Apis mellifera* L.). The guidance document (GD) on the risk assessment of plant protection products on bees (EFSA 2013) foresees the bee brood feeding test according to Oomen *et al.* (1992) as one possible test to refine the risk of potential effects on honeybee larvae. Here, free flying honeybee colonies, with access to natural food sources are exposed to one liter of a PPP spiked sugar solution, *via* in hive feeding. To investigate potential effects of a PPP, brood development of at least 100 cells containing eggs, young and old larvae is assessed in regular intervals for a period of one brood cycle (21 days) to compare the relative brood termination rate (BTR) between treatment groups.
The method once was created to detect hazards of Insect Growth Regulators (IGR`s) to bee brood in a more or less simple "black-or-white" decision. It has never been validated or ring-tested.

Aim of the Study and Method

On total of 17 studies were evaluated regarding the quality and variation of the BTR as one of the main endpoints. The studies were performed by German and Swiss laboratories over a period of 15 years. Single bee colonies were regarded as independent replicates (n=49 to 51). Fenoxycarb at a rate of 0.75 g a.i./L (≙ 300 g a.i./ha), was used as a reference item.

Results

According to the present data, no influence of the start of the experiment during the year on the BTR could be detected. Mean BTRs in the control were 23.8 % (eggs), 17.4 % (young larvae) and 7.7 % (old larvae). In the reference item treated groups they amounted to be 76.3 % (eggs), 57.9 % (young larvae) and 28.4 % (old larvae) (Table 1, Figure 1) and were statistically significant different from the control for each stage (Welch t-test, one-sided greater, p<0.001). Thus data show decreasing sensitivity of the brood stages to chemicals with the age at the time of the first exposure.

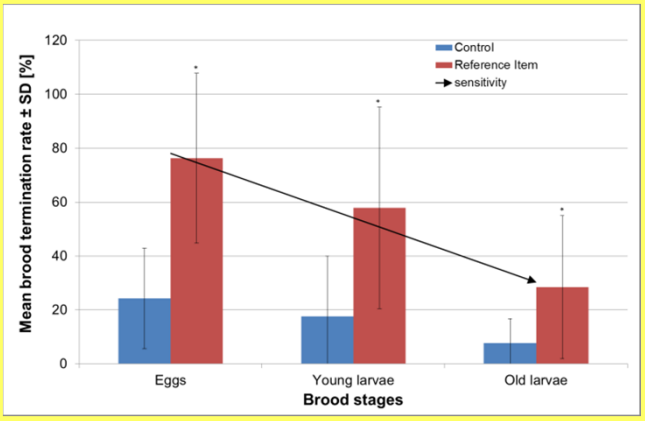

Figure 1: Mean BTRs and trend of sensitivity of the brood stages; * = statistically significant different, Welch t-test, p<0.001

Study No. / Year of performance	Termination Rate [%] [1]					
	Control			Reference Item		
	Eggs	yL[2]	oL[3]	Eggs	yL[2]	oL[3]
1 / 1997	19.9	11.4	6.8	99.8	100.0	33.4
2 / 2002	10.2	6.7	6.9	90.0	30.0	13.0
3 / 2008	5.8	3.6	2.0	42.8	49.2	3.9
4 / 2010	20.0	-	-	24.2	-	-
5 / 2011	15.6	32.5	7.9	99.8	99.8	74.8
6 / 2011	41.2	13.7	8.8	100.0	99.8	32.5
7 / 2011	31.6	33.0	6.9	85.6	20.2	1.3
8 / 2011	8.3	3.5	3.7	38.8	12.4	3.6
9 / 2011	32.0	38.0	10.4	67.5	72.2	61.9
10 / 2012	16.0	18.9	2.7	97.4	83.1	16.2
11 / 2012	18.9	14.6	3.3	100.0	97.8	54.3
12 / 2012	60.4	51.0	10.0	94.9	72.1	15.8
13 / 2012	31.7	16.7	13.7	99.7	81.7	17.3
14 / 2012	9.3	7.0	24.7	17.7	9.3	12.3
15 / 2012	25.3	12.7	1.7	64.0	14.3	10.3
16 / 2012	41.1	7.7	5.0	85.4	43.9	51.8
17 / 2012	16.7	6.7	8.0	89.8	39.8	52.7
Mean	23.8	17.4	7.7	76.3	57.9	28.4
SD	14.4	14.0	5.6	28.5	34.3	23.6

[1] = at test end on day 21-22 - = not assessed
[2] = young larvae; [3] = old larvae

Table 1: Mean BTRs in the control and reference item group; green boxes indicate minimum and red boxes maximum BTR for respective brood stage

Results

Control colonies with more than 10,000 bees displayed statistically significant more frequent BTRs <20 % than smaller ones (Fisher`s exact test, p=0.0217) (Figure 2). There was also a relationship between the BTR and the number of dead pupae found: replicates with lower BTR showed more frequently increased pupae mortality (Figure 3).

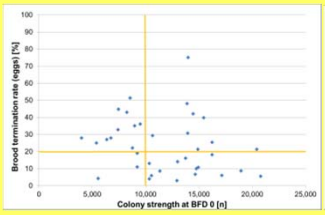

Figure 2: Colony strength *vs* BTR_{eggs}

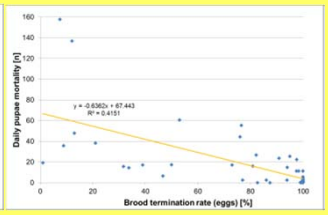

Figure 3: BTR_{eggs} *vs* dead pupae

Perspective and Conclusion

In future, new data will be collected and assessed. The presented data here, can be useful for a better evaluation of experimental results in regulatory processes. Verification of exposition of the bee brood to PPP can be either done by demonstration of a high BTR and/or high pupae mortality. Using bigger colonies are advised. Oomen tests can also be started late in the season. Last but not least the low sensitivity of old honeybee larvae to chemicals raises the question if it is justifiable to use old larvae for the toxicity-assessment of chemicals to honeybee brood.

References

Oomen P.A., de Ruijter & J. van der Steen 1992: Method for honey bee brood feeding tests with insect growth-regulating insecticides. - OEPP/EPPO Bulletin 22: 613-616.

EFSA 2013: EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). - EFSA Journal 11(7): 3295 [266 pp.].