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Single versus double field rate: Do different rates of fenoxycarb in chronic Oomen bee brood feeding tests cause different effect sizes?

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Introduction

The current EFSA "*Guidance Document on the risk assessment of plant protection products on bees*" proposes to change the Oomen bee brood feeding test (OOMEN *et al.,* 1992) from a single-day-testing to a chronic-feeding test. Based on this, the feeding period of honeybee colonies with a product-spiked sugar solution should be extended from one to nine days to guarantee chronic exposure of bee brood. However, no recommendations regarding the concentration of the reference item fenoxycarb were given. Therefore, a ring-test protocol for a chronic feeding test under field conditions was set up (for details see LÜCKMANN & SCHMITZER 2014) suggesting the ninth part of the double field rate of 300 g fenoxycarb/400 L water/ha which equals to 42 mg fenoxycarb administered in 0.5 L sugar solution/feeding day/colony. As no information were available about the dose-dependent effectiveness of the reference item, e.g. size of the brood termination rate or pupal mortality, the study presented here intended to investigate effect sizes of the single and double field rate.

Material & Methods

Two separate bee brood feeding studies following the method given by the ring-test protocol of the 'AG Bienenschutz' were conducted: one in July 2013 (study 1) and one in April 2014 (study 2). Test rates in both studies were 21 mg fenoxycarb/0.5L/colony/day ≙ 150 g fenoxycarb/ha in 400 L water and as double field rate 42 mg fenoxycarb/0.5L/colony/day ≙ 300 g fenoxycarb/ha in 400 L water. On the brood area fixing day (hereafter BFD) 200 cells either filled with eggs, young or old larvae were marked. Feeding started on the day of brood fixing in 2013 (i.e. food administration evenings) and one day after in 2014 (i.e. food administration mornings). The treated and untreated sugar solutions were administered to the colonies by feeders which were placed on the top of each colony (Figure 1). As main endpoints brood termination rate (hereafter BTR) and pupal mortality were recorded.

Data were tested on normality using Shapiro-Wilks, followed by a one-way ANOVA and in case of statistical differences by the post-hoc Tukey test for multiple comparisons, alpha = 0.05.

Figure 1: Feeder

Results

Control results show

- low pupal mortality and low BTRs (see Table 1) for all stages; BTRs were comparable to historical data of acute Oomen bee brood feeding studies (cf. Lückmann & Schmitzer 2013)

Fenoxycarb results show

- both rates caused distinct increased BTRs for eggs but very low BTRs for young and old larvae
- differences in BTRs for eggs were significant to the control for the double rate in study 1 (July 2013) and study 2 (April 2014) ($p = 0.022$ and 0.024), whereas this was observed for the single rate in 2014 only ($p = 0.009$)
- differences in BTRs for young and old larvae were not significant to the control at both rates and both years; exception: young larvae at double rate in study 1 ($p = 0.028$)
- dose-response relationship of BTRs for eggs was present in study 1 but not in study 2; this difference was not significant
- both rates caused a high and signifcant increased daily pupal mortality in both years (single rate: $p < 0.001$ in both years; double rate: $p = 0.003$ and < 0.001); a significant difference between both rates was observed in 2014 ($p = 0.028$)
- both rates caused a distinct and similar reduction of total mean number of brood cells (Figure 2 & 3)

Table 1: Summary of brood termination rates and daily mortalities

	Study 1 (July 2013)			Study 2 (April 2014)		
Mean brood termination rate [%] ± SD for	**Control**	**Fenoxycarb single rate**	**Fenoxycarb double rate**	**Control**	**Fenoxycarb single rate**	**Fenoxycarb double rate**
- eggs at BFD 21/22	17.0 ± 19.0 a	28.3 ± 5.7 ab	62.9 ± 27.6 b	8.9 ± 3.8 a	60.8 ± 17.8 b	50.6 ± 15.8 b
- young larvae at BFD 21/22	3.5 ± 1.1 a	8.9 ± 4.1 ab	10.3 ± 3.0 b	0.4 ± 0.3 a	0.6 ± 0.2 a	2.2 ± 2.7 a
- old larvae at BFD 15/16	2.3 ± 1.6 a	2.4 ± 2.9 a	3.9 ± 2.3 a	1.9 ± 2.0 a	2.1 ± 1.7 a	1.2 ± 0.8 a
Mean daily pupal mortality ± SD	0.1 ± 0.2 a	82.9 ± 18.4 b	67.9 ± 29.3 b	1.1 ± 0.2 a	161.0 ± 12.1 b	190.2 ± 12.5 c

Statistical analysis via one-way ANOVA and post-hoc Tukey test for multiple comparisons, α = 0.05; a,b,c: same letters indicate that groups are not statistically significanty different

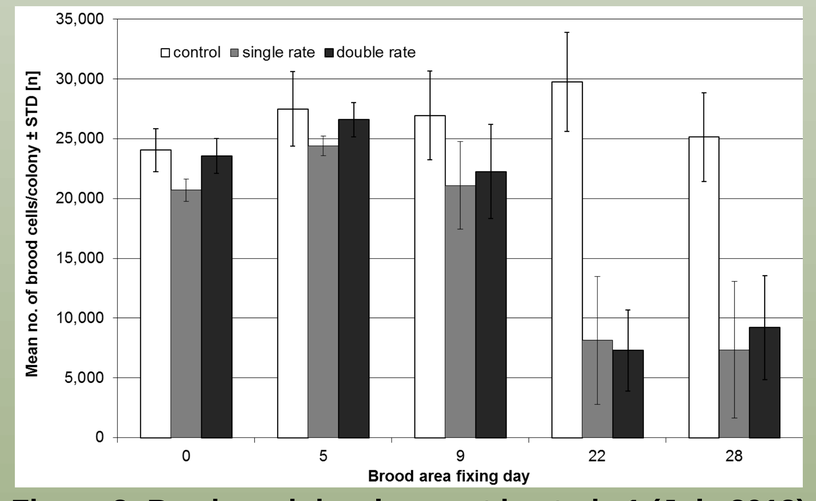

Figure 2: Bee brood development in study 1 (July 2013)

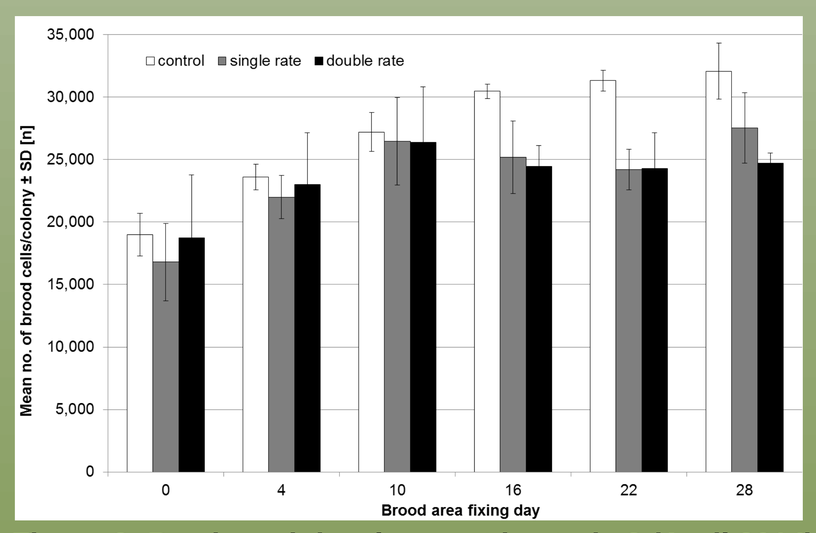

Figure 3: Bee brood development in study 2 (April 2014)

Conclusion

The chronic administration of the **single field rate** of fenoxycarb caused distinct effects on the pupal mortality and colony development, whereas a distinct (i.e. ≥ 50%) and significant effect on BTRs of eggs was observed only in study 2 (April 2014). Dose-related differences in effect sizes were present for the BTR in study 1 (July 2013) and for pupal mortality in study 2. Thus the chronic feeding of the single rate of fenoxycarb did not cause reproducible dose-related effects. Therefore we recommend to use the double field rate of fenoxycarb if this test system is applied.

Literature

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