

## **1.15 Sub-lethal effects at stake: Does the acaricide Coumaphos and fungicide Folpet affect the hypopharyngeal glands size?**

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### **Abstract**

Background: Pesticides are increasingly suspected to be involved at a global scale in honey bee decline. Most studies focus on acute effects on mortality, whereas sub-lethal effects are poorly understood. Hypopharyngeal glands (HPG), producing royal jelly to feed brood, are established marker to assess sub-lethal effects of pesticides where for example the size of the acini can be measured. The size of the later depends of different natural factors: the age of the bee and the type of task performed by the bee. The HPG are the best developed at the age of 10 days by nursing bees. Regarding the data requirements of the new EFSA bee guidance document and the recently developed OECD larva test 237 and 239 a data GAP regarding residues of PSM in the produced Royal Jelly by pesticide exposed bees which might have an adverse impact on larva development from day 1 to day 3 is recognized.

Method: The effects on the commonly and widely used varroacide coumaphos in hives and the fungicide folpet in agriculture are currently unknown. Here we measured the size of the acini of new emerged bees treated with field realistic and non-realistic doses of both substances dissolved in pollen patties fed ad libitum for nine days (N=3 cages with 20 bees in each group) and in small encaged colonies without queens. An untreated and acetone control were established. The effects of the pesticides on workers and residues in gelee royal were tested with and without brood to take into consideration variations according to the tasks performed by the bees due to labor division. . After staining HPG activity was measured as a proxy via acini size. The results will be discussed.

Results: Our results may help to improve knowledge in the development and validation of methods to evaluate the risk of bees exposed to pesticides for plant protection product authorization in an appropriate and comparable way which could be consequently implemented in standardized ring-test.

### **Introduction**

The development of hypopharyngeal glands (HPG), producing royal jelly (RJ) to feed brood, is an established marker to assess sub-lethal effects of pesticides where for example the size of the acini can be measured. Its size depends of different natural factors: the age of the bee and the type of task performed. According to the EFSA bee guidance document (2013)<sup>1</sup> observations of effects on HPGs development are recommended to cover potential effects on brood care. Sub-lethal effects on the commonly and widely used varroacide coumaphos and the fungicide folpet in agriculture are currently unknown. Therefore, we assessed the acini size and head weight of newly emerged bees fed with field realistic and non-realistic concentrations.

### **Method**

Beebread spiked with the treatments below was provided to newly emerged bees in "Liebefeld cages" either in presence or absence of young honeybee larva brood. 50 bees per cage were kept in a climate chamber. Coumaphos was diluted in acetone (solvent control 1), Folpet in chloroform (solvent control 2) and untreated pollen was used as negative control (Table 1). The acini diameters of the HPG and heads weight of the tested bees were measured after 10 days of chronic exposure to the treated pollen patties (Fig. 1).

		Treatment		
		coumaphos		folpet
		1mg/g (unreal.)	2mg/kg (real.)	2mg/g (unreal.)
Brood	+	C=3 ; B=50	C=3 ; B=50	C=3 ; B=50
	-	C=3 ; B=50	C=3 ; B=50	C=3 ; B=50

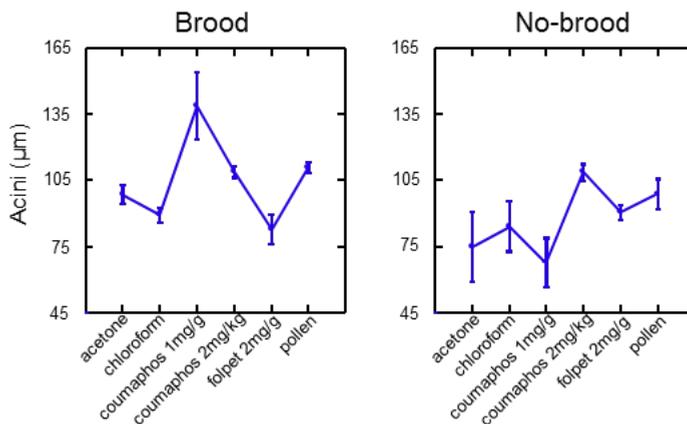
**Table 1** Tested treatments C= Nr. cages; B= Nr. bees



**Fig. 1** Acini measurements on HPG

### Results

First, the acini size is correlated with the heads weight (Pearson Correlation=0.341, p-value=0.000). Moreover, the acini size is significantly bigger in presence of brood (p-value=0.000) (Fig. 2). The quantity of pollen consumption by the bees was influenced by treatments and treatment concentrations. We observed a repellent effect of coumaphos, which was considered in our general linear model (Fig. 3). The experiment without brood is harder to interpret, as the glands have not been activated since no brood was present. For the experiment with brood, we observed that Folpet has no effect on the acini size (p-value=0.9046). In contrast, coumaphos seems to hypertrophy the acini size at 1 mg/g and 2mg/kg when compared to the solvent control 1 (p-value 1mg/g=0.0004; 2mg/kg= 0.0046).



### Conclusion

Our results demonstrate the need of additional research to determine an appropriate method for accurate and comparable results of sub-lethal effects on HPG and its functionality. Brood presence for HPG testing is crucial to cover both maturity growth and functionality growth (triggered by brood presence and RJ production) of the HPG. Therefore, to assess sub-lethal effects of pesticides on HPG, brood presence to activate the royal jelly production should be considered and included in the test method (design) under laboratory conditions.

### Reference

<sup>1</sup>EFSA Journal 2013;11(7)