

1.7 Consequences of a short term, sub lethal pesticide exposure early in life on survival and immunity in the honeybee (*Apis mellifera*)

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Abstract

Dramatic losses of pollinating insects have become of global concern, as they threaten their ecosystem services as well as human food production. Recent research provided evidence that interactions between ecological stressors are drivers of declining pollinator health and responsible for observed population collapses. We used the honeybee *Apis mellifera* and conducted a series of experiments to test for long-term effects of a single short exposure to the agricultural pesticide flupyradifurone to a second environmental stressor later in life. To do this, we exposed individuals during their larval development or early adulthood to sublethal levels of flupyradifurone, either pure or as part of an agricultural formulation (Sivanto). We afterwards exposed bees to a second environmental stressor, infecting them with the fungal gut parasite *Nosema ceranae*. We found that pesticide exposures significantly reduced survival of bees and altered the expression of several immune and detoxification genes. The ability of bees to respond to these latter effects differed significantly between colonies, offering opportunities to breed bees with elevated levels of pesticide tolerance in the future. We conclude that short episodes of sublethal pesticide exposures during development are sufficient to trigger long-lasting effects that could contribute to the widespread declines in bee health.

1.8 How does the novel insecticide flupyradifurone affect honeybee longevity and behavior?

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Abstract

Flupyradifurone (4-[(2,2-difluoroethyl)amino]-2(5H)-furanone) is a new insecticide which was recently introduced to the market by the Bayer AG (Bayer AG, Crop Science Division, Monheim am Rhein, Germany). It belongs to Bayer's own new class of butenolides and is highly effective against sucking "pest" insects, especially white flies and aphids. Similar to the neonicotinoids, flupyradifurone binds to nicotinic acetylcholine receptors in the insect brain and works as a reversible agonist.

So far, very little is known about sublethal effects of flupyradifurone on honeybees. We investigated the effect of this substance on honeybee longevity, sensory responsiveness, cognition, foraging initiation and flight behavior, behavioral rhythms and motor behavior. We analyzed both effects of acute treatment and of chronic exposure.

Interestingly, chronic application of flupyradifurone in low concentrations had no significant effect on survival of honeybees in cages of 30 individuals but significantly reduced survival of bees kept individually in activity monitors, indicating that additional stress through isolation might lead to synergistic effects. Further, in four out of eight replicates, flupyradifurone-treated bees did no longer display circadian rhythms in activity monitors compared to control animals.

When honeybees were treated chronically in the hive and their flight behavior was monitored using radio frequency identification (RFID), we measured a significantly earlier onset of foraging in the flupyradifurone group. Otherwise, flight activity did not seem to be affected.

Abstracts: Oral Presentation

Acute treatment with flupyradifurone reduced sensory responses and cognitive performance as well as motor behavior with typical indications of toxification such as walking in circles or falling on the back.

Generally, low concentrations of flupyradifurone had smaller effects on behavior than the hitherto frequently used neonicotinoids. However, we also see a negative impact of this novel insecticide on honeybees, even though it may sometimes only become apparent under stressed situations.

1.9 Dust drift from treated seeds during seed drilling: comparison of residue deposition in soil and plants

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Abstract

Drilling of seeds treated with plant protection products leads to dust drift carrying active substances (a.s.) into adjacent areas. Since these residues potentially pose a risk for bees, standardised field experiments have been conducted between 2009 and 2017 to investigate the deposition pattern of a.s. and the potential bee exposure to a.s. The large resulting data set contains a lot of information that can be used to improve our understanding of how different parameters influence the deposition pattern of dust and a.s. of seed treatments. For the present analysis, residues sampled in different matrices were used, including Petri dishes placed on bare soil and within neighbouring cultures (oil seed rape and mustard) as well as plant material (divided into flowering and non-flowering plant parts). In a nested design, multiple samples were taken at each distance of 0, 1, 3 and 5 m from the field edge within a total of 6 blocks per trial. The a.s. content per sample was determined analytically, using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS).

By means of generalized linear mixed effect models (GLMM; R package 'lme4') and automated model selection (R package 'MuMIn'), the effects of environmental and drilling parameters, seed treatment quality and sampling matrix were analysed taking into account the information from multiple trials and thus allowing for analysing the effects independently from another. A high amount of variation cannot be explained by the resulting models, probably due to environmental factors not incorporated into the models, such as varying wind speed and direction as well as heterogeneous field characteristics (terrain, crop density). However, the incorporated fixed effects resulted to be relevant in the majority of the selected models. Overall, the dust-borne a.s. emission per hectare (Heubach value expressed as g a.s./ha) has a strong impact on the amount of residues, which decrease markedly within the observed distance of 5 m to the field edge. Comparing different sampling matrices, *i.e.*, flowering plant parts and ground-based Petri dishes, a similar distance-related residue pattern was observed within the neighbouring crops. Based on field realistic data, the presented results will contribute to enabling a more precise risk assessment of seed treatment applications with regard to bees.

1.10 Coumaphos residues in beeswax after a single application of CheckMite® affect larval development *in vitro*

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Abstract

Coumaphos is an organophosphate insecticide used on bees for the control of the parasitic mite *Varroa destructor*. We studied the distribution of coumaphos in beeswax after a single application of CheckMite® and