5.6 Residues in bee-relevant matrices

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Abstract

Application of pesticides during flowering of crops can result in exposure of pollinating insects such as honey bees, bumble bees and wild bees. In addition, residues of pesticides in bee products like honey may result from such applications. One of the overall goals of the German "FitBee" project was to determine the transport of plant protection products into the honey bee colony via individual bees and reduce the exposure to plant protection products by application technology approaches. One of these application technologies is Dropleg^{UL}, with which row crops can be sprayed underneath the canopy level, avoiding spray onto the blossoms. In the scope of the "FitBee" project (2011 to 2015), we conducted during five years semi-field experiments in Germany comparing conventional and Dropleg^{UL} spraying techniques regarding their implications to honeybee colony exposure. In this context, various trials were conducted in which residues in in-hive matrices (stored nectar, pollen) of bee colonies foraging on a model crop (oilseed rape) which was pesticide-treated with Dropleg^{UL} vs. conventional technology were measured.

Keywords: FitBee, Dropleg^{UL}, Azoxystrobin, τ-Fluvalinate, Thiacloprid, honey bees, pesticide residues

Introduction

The objective of the activities was to determine the influence of conventional application technology compared to a novel application approach on the pesticide exposure of honey bee colonies, and to reduce the active substance input from treatments during the flowering period in oilseed rape by a modified application technology.

In the studies conducted we compare two application technologies, conventional spray equipment vs. Dropleg^{UL} technology in term of residue level in nectar and pollen collected by bee colonies in treated winter oilseed rape (*Brassica napus*).

Oilseed rape was chosen as a reference crop to determine the level of in-hive residues of exposed bee colonies under semi-field conditions. The plots grown with oilseed rape were treated with different compounds such as Azoxystrobin, τ-Fluvalinate and Thiacloprid in different years, applying each treatment group during flowering, using either conventional application or Dropleg^{UL} technology, at the registered application rates of the tested products. Honey bee colonies were confined on the treated plots by means of tunnels of insect-proof netting. Samples of in-hive matrices were taken in order to analyse for residues of nectar and pollen caused by the treatments.

Material and Methods

Study design

The study sites were located at the Bayer AG experimental Farm “Höfchen” in Burscheid (Germany, Nordrhein-Westfalen) between 2011 and 2015. In all studies oilseed rape (*Brassica napus*) was sown under praxis relevant conditions, between 6 to 8 months before the studies were conducted.

At the onset of bloom, small honeybee colonies were set up at the plots. In order to prevent honeybees from leaving the study plots and make sure full exposure of the bees to the treatment, tunnels of insect-proof netting (5 x 30 m) were placed on the study plots. Each tunnel containing one bee colony was defined as one test unit. The colonies remained in the tunnels for max. 15 days after application and were afterwards taken out for further assessments.

For each treatment group (i.e. control, test substance treatment with conventional application, test substance treatment with Dropleg^{UL} application) three test units was set up in the field. As test substances, we used the pyrethroid, τ-Fluvalinate as a non-systemic insecticide, Azoxistrobin as a

systemic fungicide, and the neonicotinoid, Thiacloprid as a systemic insecticide. These substances were chosen for testing as representative compounds for the described characteristics.

The test units installed over the crop before flowering began. Bee hives were set up on the test plots at least two days before application.

Honey bee colonies (*Apis mellifera*) were obtained from a local beekeeper. For the trials bee colonies were chosen without visible signs of *Varroa* or *Nosema* infestation.

Each hive had approximately 2500-3000 bees and one queen. The application of the treatment groups were carried out when the BBCH stage of the crop was 63 (30% of the blossoms open) to 65 (Full flowering, 50% of the blossoms open) at daytime during bee flight.The water spray volume in all cases was 300 litres per hectare. The following equipment was used for application:

Conventional spray equipment:

	2011	2012	2013	2015
Sprayer	Rau D2 1000 L Air Plus, 15 m spray boom			spray boom, 2 m spray width
Nozzle	IDK 120-04			TeeJet 110 02 VS

Dropleg^{UL} spray equipment:

	2011	2012	2013	2015
Sprayer	Rau D2 1000 L Air Plus, 15 m spray boom			Bicycle sprayer, 2 m spray width (DroplegUL mounted only at one boom side)
Nozzle	TwinSprayCap with 2 deflector nozzles 90° (2 x 684.406*), caliber 03		Flood nozzle 140 °, Lechler 2 x 684.406.30 per Dropleg	

Flow rate: calibration before application, documented in the raw data (300 L/ha)

Application speed was 3 km/h for conventional and for Dropleg^{UL} application.

Sampling

Pollen was collected using a pollen trap in front of the bee hives for three to four hours each sampling. Nectar samples were taken using a syringe and extracting directly from nectar cells in the combs. Sample volume was 5 ml nectar each replicate. On the sampling day, samples were finally transferred into an at least – 20°C freezer where they remained until residue analysis. Nectar sampling carried out once before application, DAT 4, 7 and 10 (±1d), at the end of the tunnel-period. Pollen sampling carried out once before application, DAT 0, 1, 3, 5, 8 and 10 (±1d), at the end of the tunnel-period.

Observations/ Biological Assessments

Foraging activity: Flight and foraging activity were assessed by recording the number of bees found foraging, using a frame (1 m x 1 m) twice per assessment in a randomized way. Inside each tunnel the observation was taken 1 min/square per assessment. Assessments carried out on DAT-2 and DAT-1 twice per day, on DAT0: 3 hours after application and once before the end of daily bee flight, then from DAT1 to removal of the bee hives from the tunnels twice per day.

Mortality: The assessment is carried out counting the number of dead bees and larvae in front of the hive and in the middle of the tunnel, where the soil was covered by plastic gauze. The numbers of dead bees were counted on DAT-2 and DAT-1 once per day, on DAT0: once in the morning, then from DAT1 to removal of the bee hives from the tunnels once per day.

Colony strength: Colony strength was determined with Liebefelder estimating methodology. Inside the tunnels these assessments carried out once before the application (DAT -2), once at the end of the tunnel period and DAT 22 (±3d).

Hive Weight: Furthermore, the weight of the bee hives was measured on the same assessment day as the colony strength, nectar and pollen stores and breeding success data was assessed.

Nectar stores: The amount of stored nectar was assessed by the estimation of the percentage of total comb area, on both sides of the comb, containing cells filled with nectar (Liebefeld method).

Pollen stores: The amount of collected pollen was assessed by the estimation of the percentage of total comb area, on both sides of the comb, containing cells with pollen (Liebefeld method).

Egg-laying activity: The egg-laying activity of the queens was assessed by inspection of the brood combs. During each inspection, the percentage of total comb area was estimated on both sides of the comb, containing cells with an egg (Liebefeld method).

Breeding success: During each inspection, the percentage of total comb area was estimated on both sides of the comb; containing egg, larvae and pupae (capped brood) (Liebefeld method). Inside the tunnels these assessments carried out once before the application (DAT -2), once at the end of the tunnel period and DAT 22 (±3d).

Results

Biological Assessments

During the entire exposure period the mean flight and foraging intensity in the test substance treated groups was similar compared to the control and no significant difference in the flight and foraging activity was observed between conventional, Dropleg^{UL} and untreated groups.

Hive weight development over the course of the studies likewise revealed no evidence of any significant differences between conventional, Dropleg^{UL} treatment and control groups.

The strength of the colonies increased during the exposure period in all treatments compared to the assessment carried out before the start of bee exposure.

The continuous presence of eggs, larvae and pupae in all colonies showed that the queens and the bee colonies were in good condition after the end of exposure. No differences in the condition of the colonies or the brood development between the colonies of the different test substance groups and the control group were noticed.

The continuous presence of pollen and nectar cells indicated that the bees visited the oilseed rape plants and that an exposure to potential residues of the treatment was ensured in the test substance treated groups. There was no significant difference between the numbers of pollen and nectar cells between conventional, Dropleg^{UL} and untreated groups.

During the entire test period the average number of dead bees in the test substance treatments was similar or lower compared to the controls. The average number of dead bees per colony was in the normal range of bee mortality that normally occurs under semi-field conditions.

Residues in Nectar and Pollen

The exposure of the test colonies was measured by residue analytical determination of the residue levels of the test substances in nectar and pollen collected by the bees. The results for τ-Fluvalinate, Azoxystrobin and Thiacloprid showed that the residue levels in nectar samples after Dropleg^{UL} spray were substantially lower than at the residue levels in nectar and pollen from the plots treated with conventional spray.

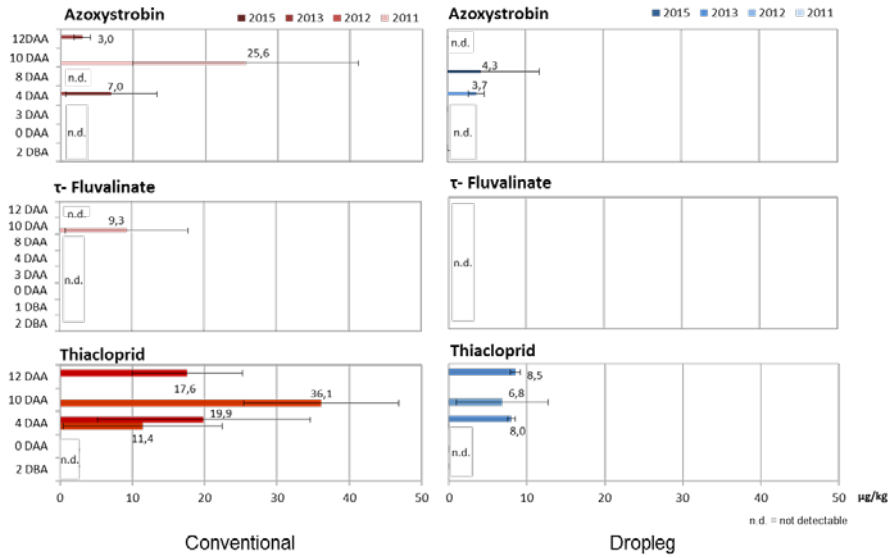

Figure 1 Average residue results in nectar from conventional vs. Dropleg[UL] application technology of three representative pesticides (τ-Fluvalinate, Azoxystrobin and Thiacloprid) during four years semi-field experiments in Germany

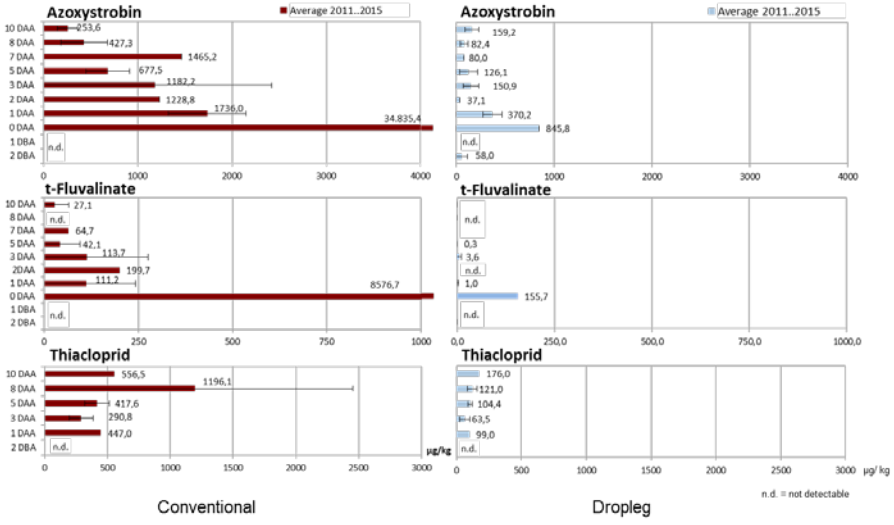

Figure 2 Average residue results in pollen from conventional vs. Dropleg[UL] application technology of three representative pesticides (τ-Fluvalinate, Azoxystrobin and Thiacloprid) during four years semi-field experiments in Germany

Conclusions

In our studies comparing conventional vs. Dropleg[UL] application technology regarding residue levels of three representative pesticides (τ-Fluvalinate, Azoxystrobin and Thiacloprid) in nectar and pollen of a treated reference crop, none of the test substances caused effects to mortality, foraging activity, colony development, and hive weight.

A clear reduction in the exposure of bee colonies to the tested plant protection products by the Dropleg^{UL} method compared to conventional application could be shown by means residue analyzes of pollen and nectar.

Very low to non-measurable (<LOQ) residue level of the test substances were measured in nectar samples from plots treated with the Dropleg^{UL} application method.

In pollen samples a clear reduction of the residues of the test substances could likewise be achieved by using the Dropleg^{UL} application method.

Therewith, it could be clearly shown that the Dropleg^{UL} technology has the potential to substantially reduce the exposure of foraging honeybee colonies to foliar pesticide treatments.

5.7 Neonicotinoids & Pollinators: Indian Perspective

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Abstract

Pollinators provide essential services in agriculture and ecosystem as a whole. The reproduction of nearly 85 % of the world's flowering plants and production of 35 % of the world's food crop depends on pollinators. In the recent years, the concern over the decline in pollinator population has gained impetus due to the decrease of plant species and vice versa. Although, the abundance of pollinators in the environment is influenced by a number of biotic and abiotic factors, the injudicious use of chemical pesticides is maximizing the damage.

Neonicotinoid insecticides have successfully controlled pests in various crops. They have zero phytotoxicity and are compatible with all relevant crops. However, they may not only affect pest insect but also non-target organisms such as pollinators. In India, neonicotinoid pesticides were first registered for use in mid 1990s. With the overall decline in pollinators and worldwide neonicotinoid use, their impact on pollinators has become a cause of concern and more accurate risk assessments are needed critically.

Neonicotinoids are currently the most widely used group of insecticides in the world comprising 25 % of the agrochemical market. They have been subjected to public debate considering their potential role in pollinator decline. A lot has been published and many opinions have been voiced but the science and facts underlying the issue have not been clearly laid out. Till date the research on the hazardous effect of neonicotinoids has been confined to the environmental neonicotinoid residue levels in crops and pollinators and sub-lethal effects to pollinator populations. Besides, research investigating the effects of neonicotinoids on pollinators is primarily restricted to honey bees but other pollinators should also be taken into account.

However, it is important to mention here that neonicotinoids are safer to animals, mammals and environment. All chemical insecticides are harmful for bees. Use of insecticides is not the only cause for decline in natural pollinator's population. Decline is due to several factors and thus effort should be laid on conservation of pollinators.

In view of the concern over the risk of neonicotinoids on pollinators, on the recommendations of the Department of Agriculture and Cooperation, Ministry of Agriculture, Cooperation and Farmer Welfare, Government of India and Indian Council of Agricultural Research agreed to conduct the two years multi-location and multi-centric study on the effect of neonicotinoids on honey bees and other pollinators under the supervision of All Indian Coordinated Research Project on Honey bees and Pollinators. The anticipated outcomes of the study will be to evaluate the impact of various neonicotinoids on different crops, growth and development of bee brood with the exposure of contaminated pollen, impact on foraging behavior and residual effects in bees and bee products. On the basis of the data generated through the various scientific trials, legitimate action for the sake of sustainable agriculture can be taken.