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A semi-field study to evaluate effects of sulfoxaflor on honey bee (*Apis mellifera*)

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Abstract

A semi-field study was conducted to investigate the effect of sulfoxaflor on honey bees and the residues in matrices after being used on cucumber. A suspension of 22% sulfoxaflor concentration was applied on cucumber at full bloom, at the rates of 75 g a.i./ha and 100 g a.i./ha, both with 2 applications at 6-day interval. Colonies were introduced into the tunnels one day after the first application, then mortality and behaviour of the test bees were observed and recorded every day. Cucumber flowers were taken and analysed for sulfoxaflor residues. The second application occurred 6 days later, then, observations of mortality and behaviour were conducted for 5 days, and then the colonies were removed from the tunnels and transported back to the apiary for 14 days additional observation. Besides, general conditions of the colonies were assessed before and after exposure, at the end of the field study. After being introduced into the tunnels, the colonies stabilized after 3 days' adaption. During the exposure period, sulfoxaflor showed lethal toxicity on bees, but the effect was less than that caused by dimethoate. After being removed from the tunnels, the mortalities of all groups fluctuated at a low level, indicating that sulfoxaflor had no long-term lethal effect on bees. During the whole exposure period, sulfoxaflor had no effect on the flight intensity of the bees. Meanwhile, sulfoxaflor had no obvious adverse effect on the strength and the condition of the test colonies. Residue analysis showed that: on the days of application, the residue of sulfoxaflor in cucumber flower was between 5.004 mg/kg and 5.832 mg/kg. After the applications, the residue of sulfoxaflor reduced gradually, until the 6th day after the first application, the residue reduced to 0.100~0.198 mg/kg, until the 5th day after the second application, the residue reduced to 0.155~0.304 mg/kg.

Key words: sulfoxaflor, negative side effects, honey bee, cucumber, semi-field trials.

Introduction

Sulfoxaflor is an insecticide that acts through a unique interaction with the nicotinic acetylcholine receptor in insects. It is an agonist of the nicotinic acetylcholine receptor (nAChR) and exhibits excitatory responses including tremors, followed by paralysis and mortality in target insects. While sulfoxaflor acts on the same receptor as the neonicotinoids, it is classified as its own subgroup (4C). The structure of sulfoxaflor makes it stable in the presence of a monooxygenase enzyme that was shown to degrade a variety of neonicotinoids in IRAC (Insecticide Resistance Action Committee) Group 4A, resulting in a lack of cross-resistance demonstrated in laboratory experiments (Sparks *et al.*, 2013).

Sulfoxaflor is classified as very highly toxic with acute oral and contact LD_{50} values of 0.05 and 0.13 µg a.i./bee, respectively, for adult honey bees (*Apis mellifera* L.). For larvae, a 7-d oral LD_{50} of >0.2 µg a.i./bee was determined (USEPA, 2010; 2013).

To investigate the effect of sulfoxaflor on honey bees under semi-field conditions, six tunnel studies have been conducted on cotton and other crops except for cucumber in US (USEPA, 2013). Sulfoxaflor is commonly applied on cucumber to control *Bemisia tabaci* (Gennadius) and cucumber could be visited by honey bee foragers. This study determined the effect of sulfoxaflor on honey bees and on the residues in matrices relevant to exposure of honey bee colonies following application of 22% sulfoxaflor suspension concentrate (SC) at full bloom. The results could add more evidence to the effect of sulfoxaflor on honey bees.

Materials and methods

Test chemicals

The test substance sulfoxaflor (22%w/W) SC was provided by Dow AgroSciences and the reference substance-dimethoate (40%w/W) EC (emulsifiable concentrate) was provided by the Institute for the Control of Agrochemicals, Ministry of Agriculture of the People's Republic of China.

Experimental field

The experimental field was located in Hengxi town, Jiangning District, Nanjing City, China. Not any crops had been planted in the field in the last two years. Before the start of the study, 12 tunnels were set up in the experimental field. The size of the tunnels was 48 m^2 (8 m long, 6 m wide). The maximum height of each tent was 3.5 m and the tent frames were covered with light nylon net with the mesh size of 2 mm (insect proof). 12 tunnels were randomly assigned to each application scenario, with three tunnels (replicates) for each application scenario.

Honey bee colonies

Honey bee (*Apis mellifera* L.) colonies were purchased from Nanjing Yuliang apiary. Twelve healthy and queen-right colonies with at least 10000 adult bees, 3 full combs with all brood stage each were used. The colonies were from one breeding line (sister queens newly cultivated in this year) in order to guarantee uniform bee material in all treatments.

No treatment against *Varroa* mites was performed at

least 4 weeks before start of the study. Furthermore, the following criteria for the nuclei were guaranteed: (a) at least 2 brood combs with all brood stages; (b) at least 1 honey and pollen combs; (c) bees are free of symptoms of nosema and other bee diseases. Hives used in the study were selected without conscious bias but any that were obviously unhealthy or damaged were not used.

Crop

Cucumber (*Cucumis sativus* L.) seeds (Jinchun 4#) were provided by Nanjing Institute of vegetables and flowers. Cucumber was planted two months before the test, and was managed according to the good agriculture practice (GAP). In order to control the downy mildew, 44% Metalaxyl-M chlorothalonil SC was used for three times (9th, 18th and 25th of June) prior to the first application of the test chemicals.

Experiment procedure

Treatments

The treatments were carried out at full bloom, in the early morning, when cucumber flowers were open. At the time of applications, the environmental conditions were recorded. The application scenarios were as the following:

- Application rate: 75 g sulfoxaflor a.i./ha, with 2 applications at 6-day interval (T1);
- Application rate: 100 g sulfoxaflor a.i./ha, with 2 applications at 6-day interval (T2);
- Negative control (tap water): applied on the same days as sulfoxaflor;
- Positive control (40% dimethoate EC, 100 g product/mu): in order to validate the test system and the exposure process, dimethoate was selected to be the reference substance and was applied on the same day with the second application of sulfoxaflor.

Sulfoxaflor, the reference substance and the tap water in negative control were all applied through spraying.

Introduction, keep and removal of the colonies

The colonies were introduced into the tunnels one day after the first application in the evening (one colony per tunnel). Each tunnel had one entrance, and the colonies were placed at the side opposite to the entrance and in direction to the tunnel. On the day of the second application, the entrances of the test hives were closed in the early morning (prior to the bee flight) and the hives were covered with a plastic sheet, before treatments. After application of treatments, the entrances were opened and the bees were released from the hives. The colonies were left in the tunnels for 11 days. One open container with water was placed into each tunnel. The surface of the water was covered with grasses to prevent the bees from drowning. Before introduction to the tunnels, the hives were maintained in the apiary (Huangmei town, Jurong city, Zhenjiang city, Jaingsu province, about 60 km away from the field site). After the exposure phase of the bees (five days after the second application), the hives were transported back to the same apiary for continuing feeding and observation.

Assessments of the toxicity endpoints

Behaviour of honey bees:

In order to collect and record the dead bees in and outside the hive, a bee trap was set around the hive entrance. And the paths in the middle of the tunnel and around the tunnel were covered with blue nylon nets. The number of dead bees in the bee trap and on the blue nylon nets was recorded every day. On the day of application, the recording frequency increased, such as 2 h, 4 h and 6 h after application and in the nightfall when bees stop flying. Besides, aggressive behaviour and other toxic symptoms were recorded daily.

Flight intensity:

Bee flight intensities were observed in the morning, noon and evening from the day after bee introduction to the day before the second application, 5 days after the second application. And on the day of application, the recording frequency increased, such as 2 h, 4 h and 6 h after application. At each assessment time the number of bees that are both foraging on flowering cucumbers and flying over the crop were counted on a square of 1 m^2 for 1 minute. In each assessment time and plot, the square (3 sites) observed was chosen randomly.

Colonies conditions and sizes:

The conditions and sizes of the colonies were assessed for three times: before and after exposure, at the end of the field study.

The following parameters were visually estimated and recorded when assessing the colonies conditions: presence of the queen (healthy, presence of eggs, presence of queen cells); visual assessment of the pollen and nectar storage area; visual assessment of the area containing eggs, larvae and capped cells. For the estimation of colony conditions, each side of a comb was divided into 32 equally sized areas. The number of areas per comb side fully and/or half covered with eggs, larvae, capped cells, pollen, nectar and adult bees, were estimated and recorded. For each comb side, the number of areas was summed up to a maximum of the equally sized area number. This was carried out for all combs of each hive. Afterwards the mean values were calculated for each hive.

The size of each colony was measured by weighing. When bees were present on the walls inside the bee hives, they were estimated accordingly and were added to the number of areas of one or more of the comb sides.

Flower collections

Cucumber flowers were taken and analysed for sulfoxaflor. Sampling started from the beginning of the test till the end of exposure. Every day, 10 flowers (about 2 g) for each tunnel were collected. All samples were timely transported to the analysis site under low temperature and were subsequently stored deep frozen at about −18 °C.

LC-MS/MS analysis of flowers

Weigh 2 g sample of cucumber flower and put the sample into 80 mL centrifuge tube, add 10.0 mL ace-