In early flowering as well as in end of flowering the number of observed troubles was not sufficient and differences could be not significant. On the contrary, from early flowering to full flowering the increased number of troubles provided consistent data. The difference in the number of troubles in foraging activity was significant between modalities.

When honeybees forage a tunnel of a limited surface (about 140 m²) for 10 to 15 days, potential effects or troubles can be observed. Extrapolation of such results would therefore suggest a risk of more important troubles when forager bees visit hundreds of hectares during 1 to 2 months.

Conclusion

This methodology was developed as a tool and a guideline in the risk assessment scheme for honey bees. It is now recommended in France[5] to assess potential troubles of all kinds of coated seed treatments and soil treatments on sunflowers.

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A methodology to assess the impact on bees of dust from coated seeds

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Introduction

During springtime of 2000 to 2003 much bee mortality were observed in France when sowing maize and sunflowers.

During 3-4 years beekeepers claim high mortality rates in their apiaries at the time of sowing maize and sunflowers, mainly during April and May. Blossoming crops or bad agricultural practices were not suspected (as there was neither rape seed crops nor other blossoming crops at this time), but only wild plants such as dandelion or flowering trees in the field hedges.

After several meetings with the Agricultural authorities in the South West of France and a review of different hypothesis, it was decided to investigate on dust seed being disseminated when sowing. As coated seeds were mainly used in this area, there was a suspicion of a possible contamination due to dust produced by coated seeds.

By chronological correlation seed dusts from insecticide coated seeds were finally suspected to induce these mortalities.

After a review of different coated cultivars sown in closed conditions it was decided to assess the effects of two modalities in agricultural and laboratory conditions.

The question was: 'Is there a possibility that insecticide dust be disseminated during sowing and contaminate wild flowers that are being foraged by honeybees?'

Experimental methods

First indoor tests were conducted with non moving sowing machines equipped with paper filters in order to catch dust that is disseminated in the air while the engine is running. Different kinds of coated seeds were then tested. Some seed released dust while others did not.

Testapi was requested and incited to carry out a test in order to investigate potential effects on honeybees of plants exposed to dust released during sunflower sowing.

A study extended to laboratory to assess potential effects on honeybees was conducted outdoors, simulating sowing of treated seeds. Assessments were conducted under controlled conditions to monitor bee exposition to foliage in small containers, similar to LD50 tests. This methodology is based on the reference of EPA guideline relative to residues on foliage.

Following previous dustiness test, two sunflowers seed varieties were chosen with insecticide coated seed treatment (Melody and LG 5660).

Two fields distant about 3 km were selected. The surface sown was 2.2 ha in each field. Application procedures were identical with cleaning of the pneumatic applicator of 4 sowing rows in both fields.

The plant species used as the receiving target of dust was *Tibouchina*, an ornamental species known for its hairy leaves that represents a worst case for this purpose, as pile on leaves facilitates dust retention.

The test design had 4 treatment groups: the two varieties, a control and a toxic standard. Application of toxic standard was done in an open space close to the laboratory.

Plants were placed in fields before the sowing started and remained in the fields for 2 days. The control group received no treatment. The toxic reference was treated with a liquid spray of dimethoate, in order to ascertain bee sensitivity.

Two tests were carried out, first with bees introduced in containers with foliage collected 2 hours after sowing and then with new bees introduced in new containers with foliage collected 24 hours after sowing.

Bees were taken from one sole and healthy beehive and distributed in the 4 groups and containers at random.

The surface in each container was covered with foliage taken from plants. The surface of foliage in the container was adapted with scissors to be exactly similar in cm². Then 20 honeybees were introduced in all boxes to be in contact with *Tibouchina* leaves. The foliage was removed after 24 hours but bees were kept in boxes for 2 more days. This made the duration of the test 72 hours.

The containers were placed in controlled conditions of about 26°C in temperature and over 60% relative humidity, there bees were fed with a safe sugar solution.

Remind that we had 4 treatment groups with 3 replicates of 20 bees in each group that makes 60 bees per group and 240 bees for each of the 2 tests. Mortality assessments were made at 4 hours, 24, 48 and 72 hours following exposure.

Results

From the raw data we calculated the average mortality in the 3 replicates of each treatment group using usual formulas in statistical analysis. These results were validated by mortality rates at 24 hours of 0% in the control and over 90% in the toxic standard.

The results on the two sunflower varieties are important as a validation of the use of this new study protocol.

	Average Bee Mortality in %							
	First test				Second test			
	4 h	24 h	48 h	72 h	4 h	24 h	48 h	72 h
Control	0	0	5	17	0	0	2	8
Toxic standard	31	100	100	100	12	94	94	98
Sunflower Melody	0	3	25	40	0	0	7	22
Sunflower LG 5660	0	2	5	8	0	0	0	5

Discussion and conclusion.

With no cross contamination possible, some lethal effects on bees were observed following the use of one treated seed and absolutely no effect for the other one. Experimental conditions were satisfactory as there was no wind at all and dust lay down around in the field. A little wind could have blown away the dust into hazardous directions. To ensure a better exposure it will be necessary to sow maize or sunflower insecticide coated seeds around plants placed in the middle of the field.

Following this first study, French authorities set up a 'dust schedule' to seed coating factories limiting the dust discharge to 4 grams per quintal (100 kg) of coated seed which corresponds to the safe variety (LG 5660) in above described test.

Since 2004 no more high mortalities have been attributed to sowing operations in France. This results should be of high interest for other European countries. This methodology should therefore have a place as a guideline in the regulation scheme in European countries.

Sublethal effects of fipronil on the ability of honeybees (*Apis mellifera* L.) to orientate in a complex maze

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Abstract

Background: The recent fipronil-based pesticide is accused by bee-keepers of causing depopulations in hives of honeybees (*Apis mellifera* L.). Behavioural effects during the flight of foraging honeybees would have been evoked. To test whether the insecticide fipronil may disorientate foragers, its impact on orientation in a maze was examined. Bees had to fly through a sequence of boxes to reach the target, which was a feeder containing a reward of sugar solution. After being trained to associate a green mark with the reward, foragers received 1 µg kg^{-1} fipronil orally and their capacity to orientate through the maze following the colour mark was tested and compared to control.

Results: The rate of foragers entering the maze, and so responding to the mark placed at the entrance, was reduced with fipronil-fed animals. Before and after treatment, 86-89% of bees equally flew through the whole path and arrived to the goal without mistakes. The rate of fipronil-treated bees finding path without mistakes decreased to 60%. Conversely, the rate of bees with unsuccessful searches for the goal notably increased with treatment (34% in treated bees *versus* 4% in control bees).