



## First results on the effects on honeybees of dust loss during the sowing of dressed corn seeds

Laura Bortolotti<sup>1</sup>, Piotr Medzycki<sup>1</sup>, Anna Gloria Sabatini<sup>1</sup>, Daniele Pochi<sup>2</sup>, Enzo Marinelli<sup>2</sup>, Alberto Masci<sup>3</sup>, Marina Montedoro<sup>3</sup>

<sup>1</sup>CRA-Research Unit for Beekeeping and Sericulture, Bologna, Italy; <sup>2</sup>CRA-Research Centre for Agricultural Engineering, Rome, Italy; <sup>3</sup>Italian Ministry of Agriculture, Rome, Italy.

### Introduction

During last years several outbreaks of honeybee losses have been reported all over Europe and in other countries worldwide. According to last researches, the most likely risk factors are bee diseased, agrochemical treatments, poor beekeeping management and climatic changes. These factors can act singularly or in synergy among each others. In Italy one of the main causes of spring honeybee losses is the release of dust through the fan drain of pneumatic seed drills, during corn sowing operations. This finding leads to the precautionary suspension of use of the four active ingredients (a.i.) registered in Italy for seed dressing: imidacloprid, clothianidin, thiamethoxam and fipronil.

In order to give an explanation to the different causes of bee losses, the Italian Ministry of Agriculture financed a two year national research project, the Apenet project, with the following objectives: to monitor a large number of hives on the national territory through a national monitoring network; to investigate the effect of the loss of a.i. during corn sowing and to improve the sowing techniques in order to eliminate dust dispersion; to evaluate pesticide effects, both lethal and sub lethal, on honeybees in relationship to environmental factors; to determine and study pathogens involved in colony losses. In this paper we report the first results on the loss of dust during the sowing of dressed corn seeds and its effects on honeybee colonies.

### Measurement of seed dust

The release of dust by corn seed dressed with the four active ingredients was measured by Heubach dustmeter (Figure 1) and compared with the amount declared by the producer. The results are summarised in Table 1. For all the a.i. the measured amounts were higher than the ones declared by the producers, but remain below the limit of 3 g/q, assumed as an acceptable threshold.



Figure 1 – Dressed seeds (left) and Heubach dustmeter (right) used for the measurement of seed dust.

Commercial dressing (active ingredient)	Declared dust amount (g/q)	Measured dust amount (g/q)
Gaicho (imidacloprid)	0,9600	1,6664
Poncho (clothianidin)	1,7700	2,1668
Cruiser (thiamethoxam)	1,3300	2,4999
Regent (fipronil)	1,1100	1,6663

Table 1 – Amount of dust in the seeds dressed with the four active ingredients.

### Concentration of active ingredients released during corn sowing and their effect on honeybee hives

The field trial for each of the four active ingredients consisted in the sequential sowing of three parcels of 1600 m<sup>2</sup>. Three series of five Petri dishes filled with nitrile acetate were placed at increasing distances (5, 10, 20, 30 and 50 m) from each parcel, to capture the a.i. released from the seed drill during sowing. The trials were repeated with the seed drill equipped with deflectors ("dual pipe deflectors", Figure 2), which deviate the dust toward the ground.

Six hives equipped with underbasket cages for the collection of dead bees were positioned along the border of the sowed parcels (Figure 3). Four control hives were placed some kilometres far from the sowed parcels.

The concentrations of a.i. collected by the Petri dishes in the trials with the four commercial dressing are showed in Figure 4 and 5, respectively for the seed drill with and without deflectors.

In both cases the amount of dust loss during sowing decreases significantly at growing distance from the sowed parcels (Spearman correlation,  $p < 0.05$ ). The presence of deflectors leads to a reduction in the amount of a.i. released during the sowing, from 0 to 60%, and statistically significant for clothianidin at 30 and 50 metres and thiamethoxam at all the distances (Mann-Whitney U test,  $p < 0.05$ ). Differences are not statistically significant for imidacloprid, while chemical analysis of fipronil released by the machine without deflectors are still in process.



Figure 2 – Seed drill equipped with "dual pipe deflector".

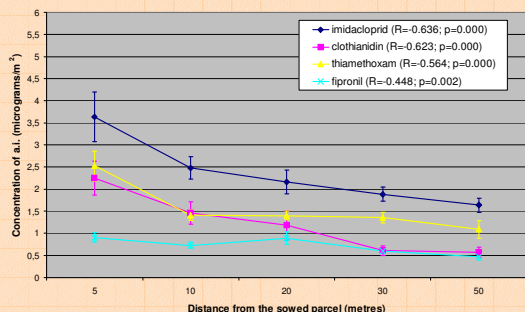


Figure 4 – Concentration of the four a.i. released during the sowing by the machine equipped with deflectors (mean  $\pm$  standard error).

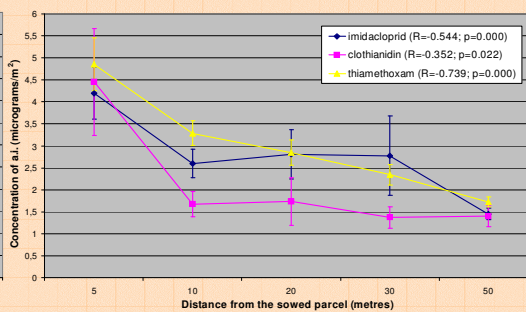


Figure 5 – Concentration of the four a.i. released during the sowing by the machine not equipped with deflectors (mean  $\pm$  standard error).



Figure 3 – Honeybee hives equipped with underbasket cage and positioned at the border of the sowed parcels.

The honeybee mortality of hives positioned at the borders of the sowed parcels, compared to that of control hives, is represented in Figure 6. In the trials with all the four a.i. the daily number of dead bees collected in underbasket cages is higher for the hives exposed to dust. Mortality is more evident about one week after the sowing and decreases about one week later, when it is comparable to that of control hives. Statistically significant values (Mann-Whitney U test,  $p < 0.05$ ) are marked by asterisks. The high standard deviations in the hives exposed to the dust indicate that mortality was not equally distributed among the different hives.

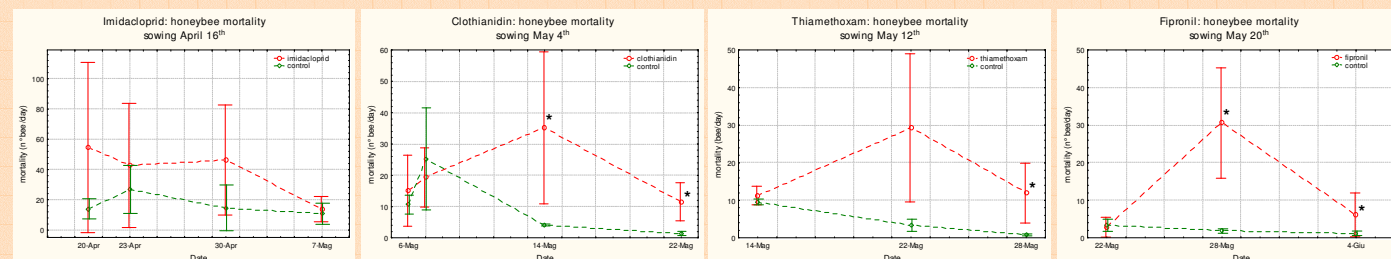


Figure 6 – Mean number of dead bees collected in the underbasket cages of hives exposed to the sowing of corn, dressed with the four a.i., in comparison with control hives (mean  $\pm$  standard deviation).

### Conclusions and ongoing researches

The results of the present researches show that the dressed corn seeds of the analysed batch have a dust loss below the fixed limit of 3 g/q; the release of a.i. during the sowing of dressed corn seeds varies between 0.5 and 3.5 micrograms/m<sup>2</sup> when the seed drill is equipped with dual pipe deflector, and between 1 and 5 micrograms/m<sup>2</sup> without deflector, with a significant reduction at increasing distance from the sowed parcel. Honeybee colonies exposed to the loss of dust during sowing show a mean daily mortality higher than control hives, especially about one week after the sowing. These results induced the Italian Ministry of Health to maintain the precautionary suspension of the four a.i. used for corn dressing also in the next growing season.

In the meanwhile, the researches of the Apenet project on this topic are proceeding. The concentrations of a.i. released during the sowing have been tested on honeybees in the laboratory, to investigate both the acute toxicity and the sublethal effects. Other possible risks towards honeybees of dressed corn plants have been investigated, like the phenomenon of plant guttation and the possible toxicity of maize pollen. The productive output of corn plants deriving from dressed and non-dressed seed have been compared, as well as the presence of soil parasites in several areas of maize production, in order to investigate the real necessity of seed dressing in different Italian regions. The final results of these researches will be available at the end of 2009.