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# Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds

**Moreno GREATTI[1], Renzo BARBATTINI[1], Antonella STRAVISI[1], Anna Gloria SABATINI[2], Simona ROSSI[2]**
[1]*Dipartimento di Biologia applicata alla Difesa delle Piante, Università di Udine, Italy*
[2]*Consiglio per la Ricerca e la Sperimentazione in Agricoltura (CRA), Istituto Nazionale di Apicoltura, Bologna, Italy*

## Abstract

In recent years the widespread use of corn seed hybrids dressed with the systemic insecticide Gaucho® (a.i. imidacloprid), has been frequently linked, by beekeepers with bee colony losses registered during the corn sowing season.

On the basis of a preliminary experiment, where the loss of imidacloprid from the pneumatic seed drills was highlighted, the aim of the study was to verify both the amount of contamination and the persistence of the active ingredient on leaves and flowers of spontaneous plants growing near corn fields in which Gaucho® dressed seeds had been used.

The observations were carried out in north eastern Italy on five different commercial corn hybrid seeds, three dressed with Gaucho® [hybrids 2 (H2), 4 (H4) and 5 (H5)], one with a new adjuvant (H5). The escape of the active ingredient from the fan drain of the pneumatic seed drill was monitored using paper filters, whereas samples of grass and flowers were collected from the borders of sown fields. Meteorological data were registered. The samples (paper filters, grass and flowers) were analyzed using a gas chromatography method.

Paper filters on the output of the drill fan have shown, at different concentrations, an escape of imidacloprid from the fan for each kind of Gaucho® dressed seed; the order of magnitude of the escape ranged on average for H2, H4 and H5 from 120 to 240 µg of imidacloprid for 1 g of paper filter at 240 seconds. Therefore the new adjuvant did not eliminate the loss of active ingredient.

Even if at different concentrations for each kind of Gaucho® dressed hybrid, imidacloprid was found the same day of sowing on spontaneous vegetation near the corn fields; H2 showed the highest level of residues with values of 123.7 and 58.2 ng/g of imidacloprid respectively on flowers and grass. Residual imidacloprid on grass and flowers was found at least four days after sowing (H2), while the washing effect of heavy rain has been observed, which seemed to hide the detection of the active ingredient (H4 and H5).

The investigation showed that Gaucho® dressed corn seeds during sowing operations can release imidacloprid into the environment, and therefore bees and wild pollinator insects could bee exposed to the insecticide molecule. Plants could accumulate the active ingredient released during different sowing operations in the same area and become polluted for a time depending on the length of the sowing period. The same problem could be extended to other pesticides, at present or in the future, used in seed dressing.

**Key words:** imidacloprid, Gaucho®, pollution, honeybees, pneumatic seed drills, corn sowing.

## Introduction

Imidacloprid, the active ingredient of the commercial product Gaucho®, is a systemic plant insecticide used for seed dressing against soil and sucking insects. The effects of imidacloprid on honeybees are well known (Schmidt, 1996; Nauen *et al*., 2001; Schmuck *et al*., 2001; Suchail *et al*., 2001a, 2001b; Maus *et al*., 2003); the active ingredient, apart from mortality, can cause a progressive decrease in the hive population and sublethal doses can determine behavioural changes (Colin *et al*., 2001; Decourtye *et al*., 2001; Guez *et al*., 2001; Suchail *et al*., 2001a; Bortolotti *et al*., 2003; Medrzycki *et al*., 2003; Colin *et al*., 2004; Rortais *et al*., 2005; Faucon *et al*., 2005).

In the last few years the extensive use of corn seed dressed with the commercial product Gaucho® has often been linked, by beekepers, with widespread bee colony losses in the period of corn seed sowing. Observations carried out in 2001 have shown that there is a loss of imidacloprid from the outflow air fan of the pneumatic corn drills and therefore possible pollution of vegetation in nearby fields (Greatti *et al*., 2003). Other investigations tried instead to quantify the active ingredient that was dispersed by pneumatic drills and verify the effect of a new adjuvant added to the seeds to reduce abrasion (Schnier *et al*., 2003; Bonmatin *et al*., 2005).

Consequently, the aims of this study were to verify the amount of contamination and the persistence of imidacloprid on spontaneous vegetation (“grass” and “flowers”) growing near corn fields in which Gaucho® dressed seeds had been sown.

## Materials and methods

The study was carried out on the experimental farm of the University of Udine (northern Italy) in April 2002.

Five different hybrid corn seeds were compared:

- hybrid 1 (H1): commercial hybrid PR34F02 (FAO class 500) - dressed with Celest® XL (a.i. Fludioxonil), Actellic® (a.i. Pirimiphos methyl) and Apron® (a.i. Metalaxyl);
- hybrid 2 (H2): commercial hybrid PR34F02 - dressed with Celest® XL, Gaucho® 350 FS (a.i. Imidacloprid) and Apron®;
- hybrid 3 (H3): commercial hybrid PR33J24 (FAO class 600) - dressed with Celest® XL, Actellic® and Apron®;
- hybrid 4 (H4): commercial hybrid PR33J24 -

dressed with Celest® XL, Gaucho® 350 FS and Apron®;
– hybrid 5 (H5): hybrid PR33J24 - dressed with Celest® XL, Gaucho® 350 FS and Apron®, with new adjuvants (not commercialised).

H1 and H2 were sown on 4th April and H3, H4 and H5 on 11th April. Each hybrid was sown in a field of about 1 hectare, the distance between fields was at least 50 meters and they were surrounded by spontaneous plant borders. The previous year fields were sown with soy-bean (H1, H3 and H5) and barley (H2 and H4). After the sowing of each hybrid the seed drill was carefully washed to reduce the possibility of contaminating the following trial. The drilling rate was about 75.000 seeds per hectare.

A Gaspardo SP 520 corn seed drill with a fan drain of 30 cm x 6 cm was used; the working pressure was about 0.06 bar and seeding speed about 4.5 km/h. The loss of imidacloprid from the centrifugal fan drain of the seed drill was monitored with laboratory paper filters (dimensions 25 cm x 25 cm, average weight 1 g, paper folded four times) (Greatti *et al.* 2003). Paper filters were placed in a cage 2 cm in front of the fan hole; therefore all the dusty air-stream was directly in contact with them but the air was partially filtered. Filters were exposed for the following periods: 30 (T1), 60 (T2), 120 (T3) and 240 (T4) seconds and for each three replications were made.

Samples of grass and flowers were collected from the borders of each field in two areas of one square meter. The samples were collected on day d-1, d0, d+1, d+2, d+4, d+6, d+8 (d0= sowing day).

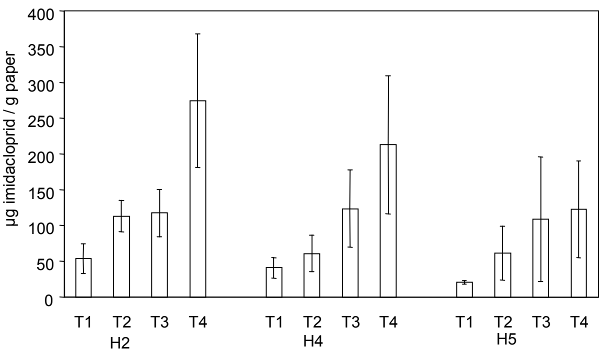


**Figure 1.** Quantity of imidacloprid (µg/g) found in paper filters in the considered exposure times for the three Gaucho® dressed corn hybrids (mean ± S.D.).

**Table 1.** Regression equations between quantity of imidacloprid found in paper filters and exposure time, and significance of the regression coefficients for the three Gaucho® dressed corn hybrids.

| Hybrid | Regression equation | P |
|---|---|---|
| H2 | y = 0.9882x + 28.384 | P < 0.001 |
| H4 | y = 0.8325x + 15.731 | P = 0.001 |
| H5 | y = 0.4466x + 28.031 | P = 0.05 |

During the period of the trials air temperature, relative humidity, global radiation and rain were recorded by an automatic meteorological station. The data were collected from 3rd to 19th April at 2 minute intervals and the daily averages calculated.

The samples (paper filters, grass and flowers) were analysed at the laboratories of the Bologna National Institute of Apiculture, using a gas chromatography method developed by Bayer (Plake and Weber, 1993), with an appropriate adjustment for the analysed matrix (Rossi *et al.*, 2005). The analysis identified 6-chloronicotinic acid as the end product of the oxidation of the a.i. imidacloprid and of its degradation products; using a conversion factor (1.622) it was then possible to calculate the equivalent quantity of imidacloprid. The detection limit (LOD) was 0.01 ng/µL and it was evaluated considering a signal three time higher the background noise. The quantification limit (LOQ) of the method used, not disregarding any of the sub products derived from imidacloprid degradation, which have a dose of toxicity themselves (US Environmental Protection Agency, 1994; Wamhoff and Schneider, 1999; Mukherjee and Gopal, 2000; Suchail *et al.*, 2001a, 2001b; Sur and Stork, 2003), was 24.3 ng/g of paper in paper filters and 4.9 ng/g of plant in grass and flowers.

## Results

### Paper filters

The amount of imidacloprid dispersed in the environment could not be calculated from the paper filters placed on the fan output though the escape of the active ingredient for each type of Gaucho® dressed corn seed was evident (figure 1); imidacloprid found on paper increased with increasing exposure time and the regressions between quantity of a.i. and time were significant for all the three Gaucho® dressed hybrids (table 1). Especially after the longest exposure times, the paper was pink in colour and often contained dust particles. In H5 the addition of the adjuvant seems to have reduced, but not eliminated, the escape of the active ingredient.

Difficulties were found with drill decontamination: even if the machine was carefully washed after each sowing operation, the values relating to sowing without Gaucho® (H1 and H3) were not zero (respectively 1.7 and 12.6 µg of imidacloprid for 1 g of paper filter at 240 seconds).

### Grass and flower samples.

"Grass" samples included both Monocotyledon and Dicotyledon leaves and stems while "flowers" consisted of headlings of *Taraxacum officinale* L. (over the 98 % of the total weight) and *Bellis perennis* L. and flowers of *Veronica* sp.

The environmental dispersion was confirmed by the presence of imidacloprid in both grass and flower samples collected in the borders of each field sown with Gaucho® dressed seeds; in contrast no residues were found in the samples picked near the fields where no Gaucho® dressed seeds were used (H1 and H3).

For each kind of seed dressed with Gaucho®, on the sowing day (d0) the presence of imidacloprid on grass and flowers samples collected has been measured (figures 2, 3 and 4). On this date residues found on flowers were about five times higher for H2 (123.7 ng/g of flowers) than for H4 and H5 (25.1 and 22.4 ng/g of flowers respectively) while residues on grass samples were similar for all three Gaucho® hybrids, with values ranging from 40 to 58 ng/g of grass. A different pattern was noticed on the days after sowing; in fact for H2 (figure 2) residues of the active ingredient were actually found at least 4 days after sowing (8.9 and 4.9 ng/g in flowers and grass, respectively) while for H4 and H5 they were only in the samples of the sowing day (figures 3 and 4).

### Meteorological data

Meteorological data registered during the period of the trial are reported in figures 2, 3 and 4. Regarding H1 and H2 (figure 2) for three days after sowing the weather was warm; from the fourth day temperatures decreased and rain was frequent. Climatic conditions in the days after sowing H3, H4 and H5 were cold and wet (figure 3 and 4), with over 95 mm of rain falling in the first 24 hours. In comparison on the sowing day of H1 and H2 the temperature mean was 16.8 °C and global radiation 20967 kj/m$^2$ while for H3, H4 and H5 the temperature mean was 8.6 °C and global radiation 7825 kj/m$^2$.

## Discussion and conclusions

The investigation confirmed, as has already been shown (Greatti *et al.*, 2003; Schnier *et al.*, 2003), the escape of imidacloprid from pneumatic seed drills using Gaucho® dressed corn seeds. Paper filters placed at the output of the centrifugal fan drain of pneumatic seed drills could be used for monitoring losses of insecticides.

The imidacloprid emission differed between hybrids; it is not known if this difference is influenced by seed surface rugosity, since residues on the filters are often abraded particles and small scales. In the case of H2, dressed with Gaucho® and already used in another trial (Greatti *et al.*, 2003), the quantity of active ingredient found in the filters was similar in both the experiments and increased with increasing exposure time.

The difficulty found in washing the drill, which continues to disperse active ingredient even when using seeds without Gaucho®, shows that a dirty drill could temporarily pollute areas in which Gaucho® dressed seeds are not used. Pneumatic seed drills in addition to emitting the active ingredient can also cause a lot of dust that could bind to the pesticide molecule and then be dispersed in the environment by wind.

Imidacloprid was found on spontaneous vegetation near fields after the sowing operation with Gaucho® dressed seeds; it was possible to quantify the active ingredient on grass and flowers and also verify the influence of climatic variables on its persistence. In fact a different pattern was noticed in the days after sowing that could be related to climatic conditions; the absence

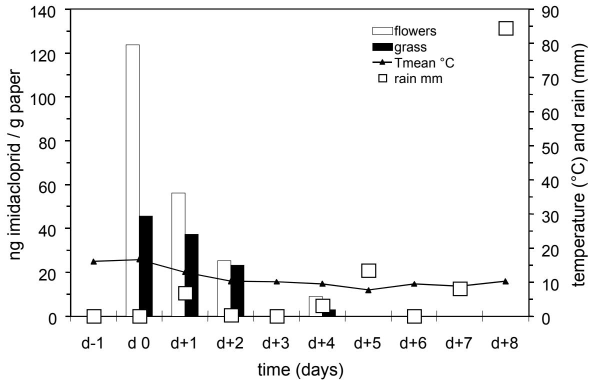


**Figure 2.** Residues of imidacloprid found on flowers and grass samples collected near the field sown with hybrid 2 (H2), rain and temperature registered during the H1 and H2 sowing period (d0: sowing day).

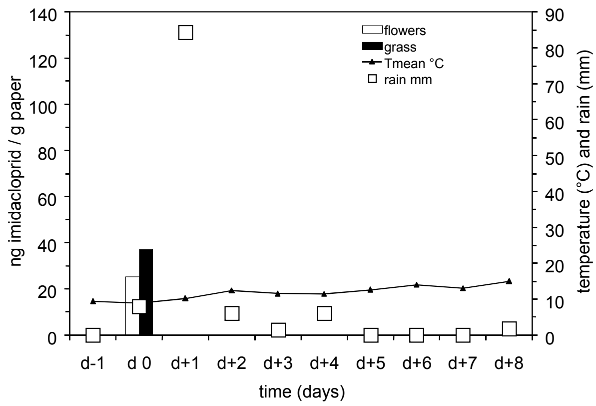


**Figure 3.** Residues of imidacloprid found on flowers and grass samples collected near the field sown with hybrid 4 (H4), rain and temperature registered during the H3, H4 and H5 sowing period (d0: sowing day).

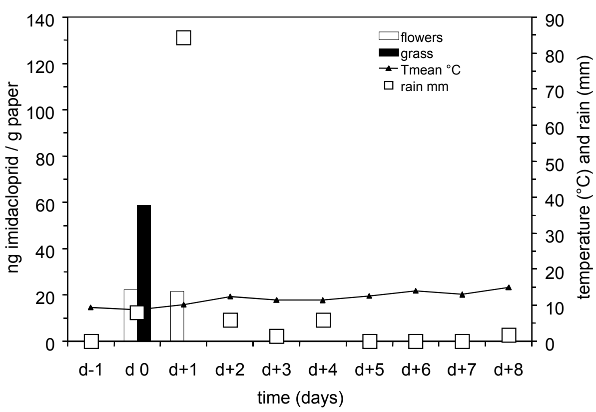


**Figure 4.** Residues of imidacloprid found on flowers and grass samples collected near the field sown with hybrid 5 (H5), rain and temperature registered during the H3, H4 and H5 sowing period (d0: sowing day).

of heavy rainfalls after sowing H2 seems to have determined a greater persistence of imidacloprid on grass and flower samples. Therefore heavy rain has a washing effect hiding the detection of imidacloprid on vegetation. Moreover the low values of active ingredient found in flower samples immediately after sowing H4 and H5 could be connected to the low temperature that did not favour the opening of *T. officinale* headlings.

The possibility that honeybees collect nectar, pollen, water and drops of dew on spontaneous vegetation and come into contact with imidacloprid is definitely realistic, especially in intense corn cropped zones considering the limited presence of foraging areas and the spring blooming during the same period. Wind and dust dispersal during sowing could greatly enhance the spread of imidacloprid in the environment. So the vegetation could accumulate the active ingredient from different sowing operations in the same district, becoming polluted for a time related to the length of the sowing operations (3-4 weeks). In extensive corn growing areas bees can come continuously into contact with sublethal and lethal doses that can cause respectively chronic intoxication with serious consequence for the hive population (Colin *et al*., 2001; Decourtye *et al*., 2001; Suchail *et al*., 2001a; Bortolotti *et al*., 2003; Medrzycki *et al*., 2003; Rortais *et al*., 2005; Faucon *et al*., 2005) and mortality, as often happens in hives located in these areas.

The investigation clearly showed that sowing corn seed is potentially polluting and could endanger bees and probably other wild pollinators if hazardous substances are released into the environment. The problem could be extended also to other insecticides used for corn dressing. To reduce the environmental impact in areas intensively cultivated with corn, it is important to improve adjuvants or make the necessary changes in the seed drills.

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**Authors' addresses:** Renzo BARBATTINI, Moreno GREATTI, Antonella STRAVISI, Dipartimento di Biologia applicata alla Difesa delle Piante, Università di Udine, via delle Scienze 208, 33100 Udine, Italy; Anna Gloria SABATINI, Simona ROSSI (corresponding author: simona.rossi@unibo.it), Consiglio per la Ricerca e la Sperimentazione in Agricoltura (CRA), Istituto Nazionale di Apicoltura, via di Saliceto 80, 40128 Bologna, Italy.

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