UNAAPI
Unione Nazionale
Associazioni Apicoltori Italiani

Unaapi's synthesis and highlighting of the Report on activities and results of the APENET Project "Effects of coated maize seed on honey bees" Year 2011

apeNet

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This report refers to results available on 6th October 2011, including 2010 results not described in the 2010 report (since obtained after its publication on august 2010). Data reported do not cover all APENET trials; some results are missing.

The original and complete report is available at:
http://www.reterurale.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/860

2010 DATA

1. Evaluation of the productive and agronomic utility of maize seed treatment and persistence in plant tissues of the active ingredients used for seed coating

1.1.1 Agronomic trials

The trials aimed to **compare the production yield** of materials deriving from seed treated only with fungicide (Celest) **versus materials deriving from the same seed coated** not only with fungicide.

The agronomic trials were set up in 20 localities, mostly situated in traditional maize-growing areas.

For the 2010 experiments, materials supplied by **ASSOSEMENTI** were utilized. A **different hybrid compared to the 2009 trials was provided**, although the same one had been asked for.

The 5 treatments under study were assayed in the framework of each agronomic trial, in a randomized block plan with 4 repetitions. For each agronomic trial, standard measurements and agronomic evaluations were performed for each of the 5 treatments under study.

Results

Statistical analyses of the data showed that the mean values of the measured parameters do not differ significantly (treated vs non-treated). However, a tendency towards a greater yield in insecticide-coated seed compared to non-coated (control) was evident in some localities.

To be precise, **of the 19 total localities, in 6 (31.5%) of them there was significant difference** in yield between one or more treatments (seed coating) and the control or other treatments. More specifically **in 2 of these 6 localities, a negative effect of one of the seed coatings was observed**. **In the remaining 4 (21%) localities**, **clothianidin had a significant positive effect** on yield compared to other seed dressings and/or the control.

Furthermore, as mentioned before, **the commercial hybrid supplied by Assosementi** for the 2010 trials was different compared to the one supplied in 2009. The substitution **may have determined a different genotype-environment interaction**, also in relation to the seed coating with insecticide**, not comparable** to what was observed in 2009.

1.1.2 Monitoring of harmful soil insects

In some of the localities, **risk maps for harmful maize soil insects were drawn up** and monitoring was carried out according to **two methods**:

1) **determination of larval populations**, plant densities, and pest attacks in plots were monitored by pheromone traps in 2009

2) **monitoring with YATLORf pheromone traps** set to respond to adult forms of the main Wireworm species and Western Corn Rootworm.

The aim of **positioning the 2010 larval traps around the positioning of the 2009 pheromone traps** was to correlate the larval population with the adults of the previous year, **in order to evaluate the forecasting ability of the pheromone trap.**

In the stations which hosted pheromone traps in the previous year, absence of insecticide treatments was guaranteed.

Results

1) Determination of larval populations

In the test plots **the number of larvae per trap was always below tolerance threshold and no severe damage from soil insects was observed**. The obtained results **confirm the findings of trials on this issue described in the past decade, including the first year of APENET experiments**.

Severe damage on the maize crop (such that yield is compromised) caused by soil insects was confirmed as a rare event; plant densities were high and the **insect attacks lower or only slightly higher than 1% of total plants.**

2) Monitoring with YATLORf pheromone traps

In the second year, apart from limited cases, the distribution of adults in the main species corresponds to what was observed in the first year. Variability among locations is high, confirming that an integrated pest management can be applied differentially according to risk levels.

The small differences in average crop yield were not statistically significant. The average crop production from insecticide-coated seed was 119.85 q/ha, while from non-insecticide-coated seed was 119.3 q/ha.

1.2 Study of persistence in plant tissue of the active ingredients used in seed coating

Results

The results indicate that the four insecticidal active ingredients studied showed a drastic reduction in levels detected in leaves, from the 2nd-3rd to the 7th-8th leaf stage, up to non-detectable levels by the stage of the 13th – 14th leaf. More specifically, Fipronil showed a drastic reduction in levels from the early plant development stages (2nd-3rd leaf), while the other 3 a.i. persisted, at this stage, at higher concentrations.

Investigations on the persistence of a.i. used for seed coating in pollen showed that in all groups studied the 4 insecticide a.i. did not reach detectable levels.

2. Effects induced in bees by contact with dust during flight over a field sown with coated maize seed

2.1 Premise

The purpose of this study was to evaluate the effect of direct exposure of bees, during flight, to the dust emitted by the seeder during the process of sowing coated maize seed. The argument put forward here is that when a bee makes repeated flights towards flowering plants and flies over plots sown with coated maize seed, it may suffer lethal poisoning as a result of the dust it comes into contact with during flight. This was tested by **using two different protocols: free flying bees and bees inside mobile cages.**

2.2 Free flying bees

Preliminary trials with bees restrained inside tulle netting cages and directly exposed to dust emitted by the seeder showed a toxic effect of this type of exposure. However, in these conditions the bees could not avoid contact with the dust by escaping from the cage. To simulate conditions closer to field conditions, a trial was set up in which bees were trained to visit a feeder and were obliged during the journey between the feeder and the hive to fly over a field sown with coated maize seed. Most of the experiments were carried out in 2009 and 2010, and in part completed in 2011

2.2.2 Results

Bees captured **at the beginning of sowing showed no symptom of poisoning and no mortality was recorded in either of the two humidity conditions**; bees **captured at subsequent intervals** and maintained in elevated humidity **showed 100% mortality within 24 h, some even within an hour after the end of sowing**, whereas those maintained in conditions of laboratory humidity showed a lower mortality rate. **The short-term results are sufficient to show the synergy between exposure to dust and elevated humidity**.

The first results of chemical analyses on dead bees in the laboratory cages **indicate mean contamination levels exceeding 500 ng/bee of active ingredient.**

In the trials with fipronil and thiamethoxam, **several hundred dead or dying bees** were observed in front of the hives, expelled **during the hours immediately following the test or on the subsequent day, with a maximum of 1000 dead bees in front of some of the hives**. **An average of more than 100 ng/bee was found in the samples of bees collected in front of the hives on the day after the test**. However, evaluations were not performed to determine the effects on colonies, which apparently showed no marked reductions in flights of foraging bees

In the trial with clothianidin 2009, 400 dead bees were observed in front of the hives **3 hours after the test,** while the number rose to **1490 the following day.** In the trial with imidaclorpride 2009 (1) bee mortality was lower (less than 50 dead bees in front of the 4 hives) while in (2) 300 dead bees were observed on the day of the trial and 500 on the following day. In the trial with clothianidin 2010 about a hundred dead bees were observed in front of the 4 hives on the day following the test.

2.3 Bees in mobile cages

The **influence of a quick dusting, simulating a single bee flight over the seeder, was assessed** by using an aluminium bar measuring 4 m length, on which, at 40 cm intervals, 10 cages containing a single bee were hung. The bar was supported by two vertical poles 2.5 m long. Exposure of bees to the dust was ensured by two operators who moved the bar at a speed of 5-7 Km/h in such a way as to intercept the dust cloud at fixed distances (0-4 m and 4-8 m). For each distance two repetitions were carried out, each of which consisted in one forward run and one return, at the same speed as the seeder. After exposure to the dust cloud the cages with the single bees were placed in the laboratory at high humidity conditions, as described above. **Mortality was recorded in the following 24 h.**

In a subsequent trial involving only imidacloprid bees were placed in the tulle cages hanging from the mobile bar in order to obtain different distances from the seeder. They were then submitted to a single forward and return run of the seeder, one group on the right and one on the left of the seeder. Of the 20 bees exposed per side it was decided to analyse only 5 of them for detection of insecticide content.

2.3.2 Results

In the trials with imidacloprid and clothianidin, bees placed at 0-4 m showed a higher mortality than those exposed at 4-8 m, while in the trial with thiamethoxam mortality rate was the same at the two exposure distances.

For all three a. i. mortality rate was significantly higher compared to bees exposed to only fungicide containing dust. It must be mentioned that discharge of the seeder is placed on the right: bees which were exposed on that side contained much higher levels of a. i. compared to bees exposed on the left-hand side.

2.4 Conclusions

When a bee travelling towards a food source **flies over a seeder that is sowing insecticide-coated maize seed,** the bee **may be exposed to a lethal dose of active ingredient**, probably even in a single flight.

This thesis is supported by trials with bees in mobile cages, which allow to determinate the exposition time to dust, while in free flying bees it is not possible to quantify the number of flights that contaminate the bee. The results also demonstrate that **the dust emitted by the seeder is sufficient to kill the bees without the poisoning effect being mediated by ingestion of contaminated food**.

A further interesting aspect to emerge from this trial is the effect of humidity on bee death. The demonstration of the fact that **bees are contaminated with doses much higher than LD50 are the results of the chemical analyses**, which revealed quantities of a. i. ranging between 200 and 4700 ng/bee of a. i.. In free flying bees exposed to abrasion-dust and found dead at the hive and feeders**, the average quantity of detected a. i. was 800 ng/bee.**

The sequence of events can thus be depicted as follows: **when bees encounter the seeder, they become dusted with a potentially lethal dose of neonicotinoid**; **if R.H. is elevated, the bees die** within a few hours, but if the air is dry, they generally survive, so that the association between pneumatic seeder, maize seed coated with neonicotinoids and bee die-off is no longer clearly evident. Once their bodies have been dusted with the product, **the bees may die close to the food source** (as observed near the feeder), **along their**

flight path or **upon their later return to the hive**. In the latter case, they are **expelled by the other bees, who may in their turn have been contaminated by the dust**.

Other problems arise in connection with the pattern of **maize-growing areas**. For example, **in the Province of Padua maize is grown on 30% of the total area.** Maize is also grown on innumerable small plots intermingled with other crops and green belt areas of various kinds. It is interesting to note that other countries (eg. Germany) have likewise experienced die-off phenomena in mixed cropping areas (Nikolokis et al. 2009), whereas **in monoculture maize cropping areas, as in France, the phenomena are less marked, since the bees are less likely to fly over the sown fields, given the absence of blossoms**. Therefore the problem of spring mortality affecting bees can be seen as linked to the fragmentation of crops and to the habitual foraging flights by bees, which in all likelihood involve flying over mixed cropping areas that include fields sown with maize.

3. PER (*Proboscis Extension Reflex*) test used to evaluate the effects of clothianidin, imidacloprid, thiamethoxam and fipronil administered as contaminated abrasion-dust

3.2 Results

The percentages of **correct responses at the different time intervals (60’, 180’, 24h) for all the a. i. at increasing concentrations**, starting from the concentration corresponding to the quantity of a. i. deposited by the seeder at 5 m (indicated with x1) increasing to x10, x100, x1000. The trials herewith described **involved bees which had survived exposure**: it must be noted that the **used concentrations caused noticeable mortality**, as confirmed by other trials in the framework of the APENET project. Exposure of groups of bees was repeated several times until a sufficient number of surviving bees to use in the behaviour tests was available.

Results showed a significant effect of treatment with all tested a. i. on odour recognition ability. The percentage of fully correct responses was significantly lower in treated bees compared to the untreated controls.

As expected, the differences mainly concerned the **ability to recognise odours 24 h after exposure**, although, for clothianidin, a significant reduction was already evident 180’ after exposure.

These data demonstrate a clear negative effect of sub-lethal doses of the tested a. i. on the ability to shape and / or recover olfactory memory. This is true both when bees were exposed to doses equal to the ones measured in the field, and when bees survived exposure to much higher doses, as may happen in the case of dust drift by wind, or when crossing in flight a dust cloud containing contaminated particles emitted during seeding.

2011 DATA

4. The monitoring network

In the framework of the Italian APENET Project, **a national monitoring network has been set up**, composed of surveillance modules, with at least one module for each Region and Autonomous Province. Every module consists of 5 stations (apiaries), each of which is in turn made up of 10 hives, located in representative geographic areas of each Region. To date, the network is composed of 20 modules, 94 apiaries and 940 hives.

The function of the monitoring network is to **gather information on the health status of the bee colonies contained within the modules, by means of periodic surveys and subsequent laboratory analyses performed on the different matrices collected** (dead bees, live bees, brood, wax, pollen). In addition to routine analyses, special surveys, sample collection and analyses should be carried out at any time if abnormal mortality is reported.

On the basis of the APENET network **winter mortality in 2010/2011 was estimated to be 22.48%** (78 dead colonies on 347). Winter colony losses estimated by means of the Coloss European network questionnaire were 13.44% (1850 colonies on 13770).

2009 results showed **endemic spread of the fungus (Microsporidia) *Nosema ceranae* throughout all Italian regions**. This fungus has almost completely replaced the species previously present (*Nosema apis*), with the exception of one apiary in the province of Bolzano, where both species were detected. Thus the investigation confirmed the first reports that date back to 2007 indicating the presence of *N. ceranae* in Italy as well and allowed a clearer picture of the spread of this pest over the different areas of Italy.

The samplings carried out in 2010 in the APENET network confirmed the presence of *Nosema ceranae* only.

In the samples collected in 2010, t**he same viruses as the previous year were detected with the addition of KBV and IAPV**, the latter found in 3 apiaries in Sardinia, Lazio and Tuscany. Of the378 samples analysed in 2010, 12 resulted negative, while the prevalence of each virus in the remaining 366 samples was 96% for BQCV, 78% for DWV, 60% for SBV, 29% for ABPV. With the exception of AIV, which was never detected, the prevalence of the other analysed viruses was below 10%.

It is important to note that **this is the first nation-wide investigation based on biomolecular techniques undertaken in Italy to examine the presence of bee viruses**. Previous studies, which date back to a considerable number of years ago, were not only limited to just a few regions, but were also based on electron microscope and serologic methods, which at that time were the only techniques available to test for the presence of these pathogens.

The new knowledge acquired on bee virus distribution is of considerable interest and represents a **valid starting point for further research**.

The **chemical analyses carried out on wax and bee samples** tested for residues of organophosphate, organochlorurate, pyrethroid, carbamate and neonicotinoid pesticides. In both kinds of samples **residues of pesticides were revealed quite frequently. In wax,** which is known to “accumulate” residues, **the incidence was around 43%, while in bees** the presence of pesticides **was revealed in 12% of the samples**.

The lower frequency could partially explain the good health status of the observed colonies. It is notable that the **active ingredients most present are those with an acaricidal activity**, used for control of the mite Varroa destructor (whether registered or not).

As far as pollen is concerned it is interesting to observe that there is a **fair presence of samples containing residues of pesticides (about 27%). Compared to bee and wax samples, in pollen there is a higher percentage of residues of active ingredients not connected to beekeeping activities. More specifically, residues of neonicotinoids were found in several samples.**

4.1 The reporting system

The monitoring network is further supported by the important tool of the reporting system, which makes it possible to notify the authorities of abnormal events occurring in hives even if the hives in question do not form part of the network.

In the spring of 2008 all 185 of the reports proved to have been concomitant with maize sowing, and of the 132 samples gathered and analyzed, 57.5% tested positive for the neonicotinoids used in maize seed coating**.**

In 2009 three cases were reported, all linked to non-authorized utilization of coated maize seed.

With regard to the spring of 2010, reports did not involve maize-growing areas.

Analagous to the previous year, in spring 2011 no report came from maize-growing areas.

It is important to note that the APENET project was officially terminated at the end of March 2011, together with the associated reporting system. The subsequent reports are thereby fruit of voluntary service.

5. Determination of the minimum level of dust dispersal during coated maize seed sowing with modified seeders and estimated effects on bees

PART A: Static trials aimed at **establishing a method for evaluating the efficiency of reduction of abrasion-induced dust** and **experimental assessment of a dust reduction device prototype** devised by CRA-ING

5.1 Introduction

Among other things, CRA-ING aims **to develop appliances for precision maize seeders which will reduce abrasion-induced dust dispersed during sowing of coated seed.**

The aims and the time-frames proposed for the two projects for the studies concerning coated seed were the following:

1. Determination of the minimum level of dust released by modified seeders during maize sowing technically obtainable by applying modifications to the seeders.

2. Evaluation of sublethal effects on bees of the concentrations determined in point 1.

5.2 Performed activity

- Optimisation of the system for fixed point tests set up in 2010 (artificial wind, simulated soling) to quantify the amount of dust released by seeders with and without modification.

- Set up of two prototypes of modifications which can be applied to the seeders in order to reduce the amount of dust released into the environment.

- Analyses and data processing for evaluation of the modifications and for the estimation of the dispersal theoretically obtainable in the field with the same wind conditions.

- Communication of the concentration levels to the colleagues in charge of the experiments on the bees.

5.3 Materials and methods

5.3.2 Seeder machine used in the trials

A Gaspardo Magica six-row precision pneumatic **seeder** (planting layout 0.75m x 0.18m, 75000 seed /ha) (Figure 9-A), **was used, as for the 2010 trials.**

Prior to use in the experiments performer by CRA-ING (begun in March 2010), **the machine was subjected to trials by the Julius Kuhn Institut di Braunschweig (Germany) (JKI method) which certified that the seeder equipped with deflectors reduced dispersal of a tracking dust (brilliant- sulfoflavina) by at least 90% compared to a MONOSEM seeder.** The MONOSEM seeder represents the reference machine for calculation of seeder dust dispersion with the JKI method.

It is useful to note that **in the APENET fixed point and field trials, performed in 2010, the dust abatement obtained by applying deflectors was measured to be on average 50%, ranging from 30% to 70% reduction, according to the considered a.i..**

Furthermore, the concentrations of a. i. measured at ground level (5 meters from the edge of the field), during the field trials with the modified seeder, were found by the Units performing toxicity tests on honey bees, to cause sublethal effects.

It can thus be stated that the 90% dust dispersal abatement level certified with the JKI method is not sufficient to ensure harmlessness to bees.

Following results from the 2010 trials, activity was undertaken to increase the seeder dust dispersal abatement efficiency. To this aim two prototypes were built, applied to the Gaspardo-Magica seeder and tested in the fixed point test.

5.3.3 Seed

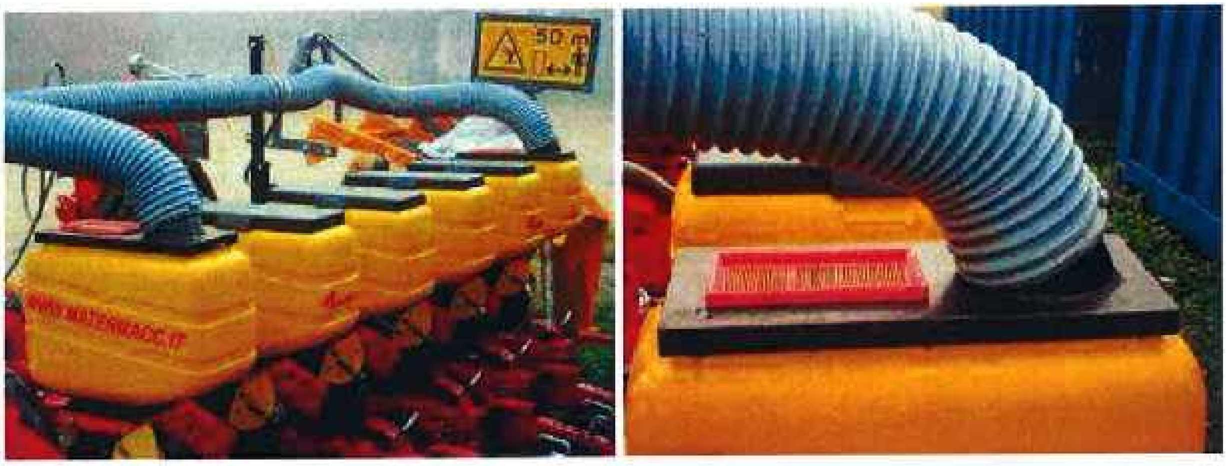
Seed was delivered to CRA-ING between 3rd-30th March 2011, thus causing delay in the beginning of the experimental activity. However, trials were initiated prior to delivery of the 2011 seed using unopened and perfectly conserved batches of imidacloprid coated seed left over from the 2010 trials.

This decision was taken considering the fact that at the beginning of the 2010 experiments, trials were undertaken to assess the dustiness of the 2009 seed

5.3.4 Seeder modification prototypes devised by CRA-ING

CRA-ING developed two alternatives aimed at increasing the abatement power of the air deflectors described in the 2009 and 2010 reports. **One alternative** is envisaged for use on seeders already in use, **by applying limited modifications. The second is envisaged for new seeders**, wherein the modification is applied by the machine manufacturer.

The principle is **to maintain the dust containing the a. i. inside the machine** by a system of air recycling and by equipping the system with openings and appropriate filters that allow exit of the air in excess without the a. i. contaminated dust.



Prototype 1 of CRA-ING applied to MaterMacc seeder used in APENET 2009 trials. An analogous prototype was applied to the Gaspardo Magica seeder, adapting it to the different air deflectors. This prototype was devised as modification which can easily be applied to existing seeders.

Prototype 2 of CRA-ING applied to the Gaspardo Magica seeder. The filter used in this prototype is a common activated carbon anti pollen car filter. Prototipo 2 del CRA-ING applicato alla seminatrice Gaspardo Magica. Il filtro qui impiegato è un filtro automobilistico (filtro antipolline a carboni attivi per abitacolo). Questa soluzione è idonea ad essere applicata su seminatrici nuove da parte del costruttore.

5.4 Methods

5.4.4. Processing of the test results

The test results refer to a theoretical sown surface of 6666.67 m2, in which the sampling area is 4.5 m wide and 22.5 m long. **The expected amounts of a. i. are very high in these conditions, and this is one of the reasons why tests on bees were not envisaged in these conditions, as they are not comparable to the real field exposure.**

5.5 Results

5.5.1 Fixed point tests

The system used is based on the identification of a protected area where artificial wind conditions are created by a fan. The seeder, raised from the ground, simulates a sowing on-site. Fan and seeder are driven by electric motors with rotation speed set on inverter mode. The area downwind constitutes the sampling area, for the measurement of deposition on the ground and of the active ingredients air concentrations. The arrangements adopted in setting up the trial system gave **satisfactory results both as regards the facilities and the equipment utilized**, and proved to be repeatable throughout tests.

5.5.2 Efficacy evaluation of the filtering material and physical characterisation of abrasion dust

The ability of the type of filter used in the prototype to retain dust by abrasion and a.i. has been evaluated though preliminary specific tests. Overall, the abatement ability measured either gravimetrically (total dust) and analytically (a.i.) was 97%. The filter was effective throughout the size spectrum considered. With decreasing particle size, the percentage of not retained dust in the filter increases (up to a maximum around 18.5%). **It should be stressed however that the finer dust particles are quantitatively less relevant (in terms of mass). On the other hand, due to their nature, they tend likely to longer persist suspended in the air and are therefore more subject to drift.**

5.5.3 Observations on dust drift

Despite the delay in arrival of the coated seed batches, the simulated soling trials were completed for all a. i.. and for all the seeder configurations described in point 5.3.4. As a result of the delay, the analyses results are, at the time of writing, only partial. They are however sufficient to yield interesting results on the abatement level which can be obtained. The available results concern tests with all four a.i. with prototype 2. While all data from the imidacloprid trials are available (due to the earlier start of the trials). These trials were useful for optimisation of the modifications.

The results were variable, starting with lower abatement values with imidacloprid (60%), due to the mentioned modifications, to progressively high percentages reaching values close to those expecting, as in referral to trials described in point 5.5.2.

The comparison between this result and the high abatement values measured in the estimation of the FAP filter efficacy, induced a specific search of possible dust dispersal from other parts of the seeder. **It was thus revealed that significant amounts of dust were dispersed through a gap that formed in the cupling edge between the deflectors and the centrifugal ventilator nozzle**, due to deterioration of the original manufacturer's sealer (silicone). **Moreover, a certain amount of dust was dispersed through the**

inspection windows of the six seeder elements. These points **were sealed**, and by repeating the tests and analysing the data it was possible to determine the amounts of dust and a. i. previously dispersed through these gaps.

Data analyses (based on the integration of regression curves on the distance) showed the **following abatement levels: clothianidin: 74.4%; thiamethoxam: 88.6%; fipronil: 94.8%.**

It is interesting to note that, as observed in the 2010 trials, **the amount of dispersed clothianidin was much higher compared to the other a. i..** This was visible to the naked eye from the amount of red dust present on the ground in the trial area, even at the greater distances. This phenomenon could be due to the higher quantity of a.i. per seed used in clothianidin coating, or to the different behavior of clothianidin in relation to the coating treatment, or even to a lower dust abatement capacity for clothianidin. These aspects will be the subject of future study.

Air concentration

The amounts of a. i. detected on the filter discs applied to the air samplers, related to the volume of sampled air and its density, provided the a.i. air concentration (ppb). The reduction resulted of 62.93% and 46.04%, respectively for prototype 2 and prototype 1. When corrected as explained above, the reduction rises to 72.44% and 53.13%. The graphs show that values do not decrease with distance, suggesting a **tendency of the dust to persist and drift suspended in air.** This is in agreement with results of the dust characterisation tests that show the **presence of a very fine fraction, not captured by the used anti-pollen filters.**

5.5.4 Forecasting field concentrations

Two examples of forecasting ground level concentrations for clothianidin, thiamethoxam and fipronil, calculated from the values measured in the fixed point tests and taking account of the sowing's width, the test duration, speed and number of passing (virtual). The concentration values are compatible with those measured in the field tests in 2009 and 2010. Integrating the regression plots with the distance from the sowing area, **the quantity of a. i. that is dispersed on a hectar of ground contiguous to the sowing area can be calculated.** The reduction of dust concentration linked to use of prototype 2, was similar to the one indicated in point 5.5.3.: 85.9% for imidacloprid, 78.3% for clothianidin, 88.4% for thiamethoxam, 91.5% for fipronil.

5.6 Conclusions

1. **The filters used in the trials showed a marked efficiency in capturing dust and the substances it contains (up to 97% of the a. i.). The ability to capture the dust decreases with dust size.**

2. **The modifications devised by CRA-ING significantly increase dust abatement of the air deflectors.** Although complete results are not yet available, the preliminary data show that **for prototype 2 the dust abatement at ground level increased from 50% with the air deflectors (2009 and 2010 trials) to 74.8% for clothianidin, 88.6% for thiamethoxam, 95.4% for imidacloprid and 94.8% for fipronil**. With the exception of imidacloprid (for these trials considerations made in point 5.5.3 apply), the above values were obtained in the reported order, showing an improvement of dust abatement linked to ongoing

improvements to the prototype. It is therefore likely that in future trials a levelling of dust abatement at the higher levels may be obtained for all a. i. (with special reference to clothianidin).

4. Use of the modifications caused the same level of reduction of dust concentration in the air and at ground level, with the same reduction percentages. **It is important to note however that the finest dust fraction is not captured by the filters used in the prototypes**. Observing the concentration trend according to distance from source, **it appears that the dust persists in the air and is able to move across a great distance.**

The presence of this dust fraction in the air gave rise to the need of measuring the quantity of a. i. met and captured by a bee in flight in an area where abrasion dust is present. To this aim, various Units participating in the project planned an experiment in which a 4 hectar plot was sown using the seeder equipe with prototype 2. In the course of the trial bees are exposed to the dust cloud formed during the sowing operations. Bees are then analysed to determine the amount of a. i. captured by their bodies, in relation to certain parameters and reference values (lethal and sub-lethal dose). The field sowing test also has the aim of evaluating functionality of the seeder with prototype 2 (uniformity of seeding) in field conditions.

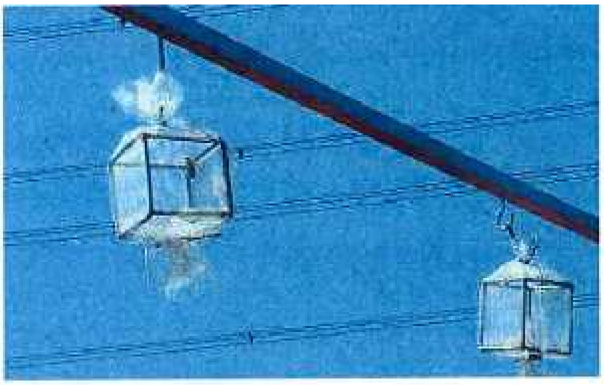
5. **The solutions proposed by CRA-ING are prototypes which are not commonly used in real field conditions.** The low dust dispersal concentrations obtained with these prototypes represent the current lowest possible concentration which can be technically reached. If these concentrations prove to be innocuous for bees, thereby gaining the status of threshold levels that must not be exceeded, **the next step will be to develop a strategy to equip all seeders with modifications (either the ones here described or others equally efficient), also considering the enormous number of seeders in use in Italy**.

6. **Application of the modifications described in these studies entails handling the spent filters which need to be periodically substituted.** The result is that **the problem of dust dispersion is shifted from a non controllable environment (the field) to a controllable one**.

8. The last consideration concerns the fixed point test system**. If a decision is made to introduce modifications with the aim of reducing abrasion dust dispersal, it will be necessary to establish criteria to assess their efficacy, on the basis of reference parameters obtained with the APENET trials.** If a decision is made to certify the above devices, the assessment procedure must be modified in order to make it faster, more economical and more suitable for routine evaluations, while maintaining the following essential characteristics: it should produce a result that can be expressed as concentration of a. i. which may be compared to reference values; the test should be carried out with coated seed; the substance used for seed coating must be harmless to operators involved and easily revealed (also by non chemical methods).

5.8.3 Bee flight trials

Forager bees of uniform age and size were selected and placed in small cages. Bee exposure occurred during two complete runs of the seeder (back and forth), according to methods described in point 2.3.1.



Cages containing one honey bee each, used in the flight trials.

Experimental group A: the horizontal bar which supports the 10 cages at 2.5 m height is fixed to the posterior part of the seeder. The picture on the right shows the system during the seeding.

Experimental group C: the bar that supports the 10 cages is kept at 1.8 m height by two operators. They proceed at 4 m from the seeder, laterally, so as to intercept the dust cloud shifted by the wind.

5.9 Results and discussion

The application of the prototype to the seeder determines a considerable improvement compared to the same seeder equipped only with deflectors. However, negative effects on bees still occur, due to the amount of a. i. which escapes action of the prototype. **A complete abatement (100%) probably represents a utopistic goal**, and **it is likely that bee mortality will continue to occur even with very low percentages of dispersed dust.**

6. Sub-lethal effects of neonicotinoids and fipronil on learning and memory of odours and spatial orientation

6.1 Introduction

Neonicotinoids are acetylcholine antagonists, as they bind to the nicotinic receptors of this neurotransmitter, causing its persistent activation and **inducing hyperexcitation, followed by death.**

Phenylpyrazols, such as fipronil, bind to the ionic pumps that are activated by gamma-aminobutyric acid, interfering with the functioning of the latter and causing, as above**, hyperexcitation and death.**

LD50 calculated for the active ingredients under study is very low (on the order of nanogrammes per bee) **and different values are reported in the various different studies available in the literature: this is due to the variable detoxification capacity of the molecules depending on the colony in question.**

Neonicotinoids and phenylpyrazols are systemic insecticides, which penetrate into the plant and can be found in pollen and nectar produced during the flowering period (EPA 2003).

Some studies found no negative effects on bees because only death from acute toxicity was considered (Nguyen et al., 2009). On the other hand, **many other studies examined more suitable methods for evaluating the risk** faced by bees that come into contact with neonicotinoids and phenylpyrazols (Desneux et al., 2007).

Such studies include toxicity tests on in-vitro reared larvae (Aupinel et al., 2005), tests based on the proboscis extension reflex (PER) to assay their effects on bee learning ability and memory formation (Decourtye and Pham-Delegue, 2002), various other behavioural tests (Thompson, 2003), and studies on the effects induced by chronic exposure, which is a concrete risk associated with the systemic nature and persistence of the above cited insecticides (Suchail et al., 2001; Decourtye et al., 2005; Ailouane et al., 2009).

Chronic poisoning

In a study on 10-day chronic poisoning, all metabolites of imidacloprid showed equal toxicity towards bees at doses ranging from 3,000 to 100,000 times lower than the dose necessary to produce the same effects by acute poisoning.

Ingestion of nanodoses of imidacloprid or of one of its metabolites for 8 days, for a total of barely 0.1 ng/bee, caused the death of 50% of the bees (Suchail et al., 2001). In other studies conducted for 10 days, LD50 by chronic oral poisoning was observed after cumulative ingestion of 0.1-10 ng /bee (Suchail et al., 2000, 2002, 2004; Guez et al. 2001, 2003), in relation to the exposure protocol (Bonmatin et al., 2005a, b).

An in-depth study (Bonmatin et al., 2003a) found that mean concentration of imidacloprid in dressed maize leaves, flowers and pollen was 4.1, 6.6 and 2.1 microgrammes/kg, respectively. Taking into account that **maize pollen represents 20-40% of the protein requirements of a beehive, consumption of 6 mg/day of pollen exposes bees to an extremely elevated 10-day chronic poisoning mortality risk** (Bonmatin et al., 2003b, 2004).

The use of neonicotinoids for maize seed dressing leads to soil contamination (NTPN 1998, Bacey 2001); in addition, it has also been demonstrated that subsequent crops and weed species may in some cases be contaminated for as long as two years after maize sowing (Bonmatin et al. 2002, 2003c).

Studies have also been conducted on fipronil. Its metabolites (the sulfonated derivative and the desulfated product, resulting from photodegradation) have been found to maintain extremely elevated insecticidal efficacy, remaining as effective as the starting molecule (tests performed on the household fly). Consequently, they contribute to the overall efficacy of the insecticide in guaranteeing prolonged crop protection (Hainzl and Casida 1996). **Dust dispersion and deposition during sowing allow the process of photodegradation, with the formation of metabolites having insecticidal action.**

Sub-lethal effects on cognitive processes (learning and memory of odours and spatial orientation)

While the concentrations specified in the above cited studies cause mortality at extremely low doses, on account of chronic repeated exposure, intake of even lower doses (or for shorter time periods) of the active ingredients under study here can still lead to effects on bee physiology and behaviour. A vast bibliography with numerous in-depth studies has been built up on these aspects (Erber et al., 1975a, b; Sandoz et al., 1995; Gerber et al. 1998; Lambin et al, 2001; Pahm-Delegue et al., 2002; Decourtye et al, 2004; El Hassani et al.2008; Maccagnani et al., 2008).

Study of the proboscis extension reflex in presence of odours associated with administration of sugar solutions allows an examination of the impact of insecticides on some cognitive processes, such as learning and memorisation of different types of environmental stimuli (Decourtye and Pham-Delegue, 2002; Maccagnani et al., 2008).

It follows that impaired odour-associated learning can be taken as an index of disruption of cognitive processes, which can severely affect bees' capacity to fulfil their foraging functions and can lead to dangerous disorientation.

For fipronil, an effect was demonstrated using doses ranging from 0.075 to 0.15 ng/bee/day, which represent, respectively, 1/80 and 1/40 of LD50 according to Chauzat et al. (2006).

The 2009 experiments conducted in the framework of the APENET project, provided evidence that **the quantity of insecticide contained in dust dispersed by the seeder and deposited on the ground at a distance of 5 m was sufficient to induce an adverse effect in bees that repeatedly came into contact with the substance.** Affected bees showed **reduced ability to recognise odours, difficulty in spatial orientation and a reduction in the function linked to foraging activity.**

Experiments were conducted in 2010 on the effects that ingestion of very low doses of the above-mentioned active ingredients can cause on learning and odour memory and on orientation in a simple labyrinth. These experiments **demonstrated:**

1. for all the active ingredients in question, **impaired ability to recognise odours** associated with the reward, and this impairment was observed both in the case of typical flower odours (citronellol) or the odour of the Nasonov gland or a component of the queen pheromone (see 2010 report);

2. a significant **reduction in colour recognition ability**,

3. for clothianidin (so far, the only active ingredient studied) it was demonstrated that **a single administration of the active ingredient at the dose of 0.7 ng/bee impairs the ability to return to the nest**, and at 0.47 ng/bee the treated bees succeed in returning to the nest but they are unable to perform the foraging functions satisfactorily.

The research programme to be set up for the spring of 2011 was planned to evaluate the following aspects:

1. The effect of contact contamination due to dust dispersion;

a. the effect induced in learning and odour memory (PER test) by exposure to 90-95% lower quantities of dust than occurred with the unmodified machine, as a result of the introduction of a new deflector prototype developed by CRA-ING, which significantly reduces dust dispersion (study completed).

b. the effects of dust-derived contamination on orientation ability in a simple labyrinth (study begun).

2. The effect of ingestion of nanodoses of clothianidin on orientation ability (return to the hive). Since no data are available on concentrations present in nectar and pollen of weed species contaminated during the sowing procedures, we used increasing sub-lethal doses according to various different protocols, in order to assess their effects on orientation ability (study completed for the clothianidin administration protocol).

Doses per bee to be administered by ingestion were established by starting out from the bibliographical data referring to oral LD50, and by applying successive dilutions until a dosage was reached that did not impair bee viability and motor ability.

The quantities of clothianidin administered to bees by ingestion (from 0.092 ng/bee to 0.552 depending on the treatment protocol) are comparable to the quantities used in acute toxicity studies and, in our view, also comparable (or at least of the same order of magnitude) to quantities taken in through the cuticle, as detected in studies on the effect of contact with dust on odour learning (PER test) (3.31 ng of clothianidin in a small cage containing 10 bees).

6.2 Effects on learning/olfactory memory caused by contact contamination with dust having reduced neonicotinoid and fipronil content - PER test

6.2.2 Results

Bees entered into contact with dust containing 10% of imidacloprid, thiamethoxam and fipronil. Although this was a lower percentage compared to the quantity dispersed by the unmodified seeder**, it nevertheless impaired odour recognition** as early as after 60' (**short-term memory**) and at 180' (**medium-term memory)** as well as at 24 h (**long-term memory).** A similar result was observed for bees that had entered into contact with dust containing a maximum of 20% of clothianidin, compared to the values obtained with the unmodified seeder.

6.3 Effects of contamination with thiamethoxam-containing dust on orientation ability in a simple labyrinth and on colour recognition

6.3.2 Results

The experiment has only just begun and 4 bees have been submitted to the treatment.

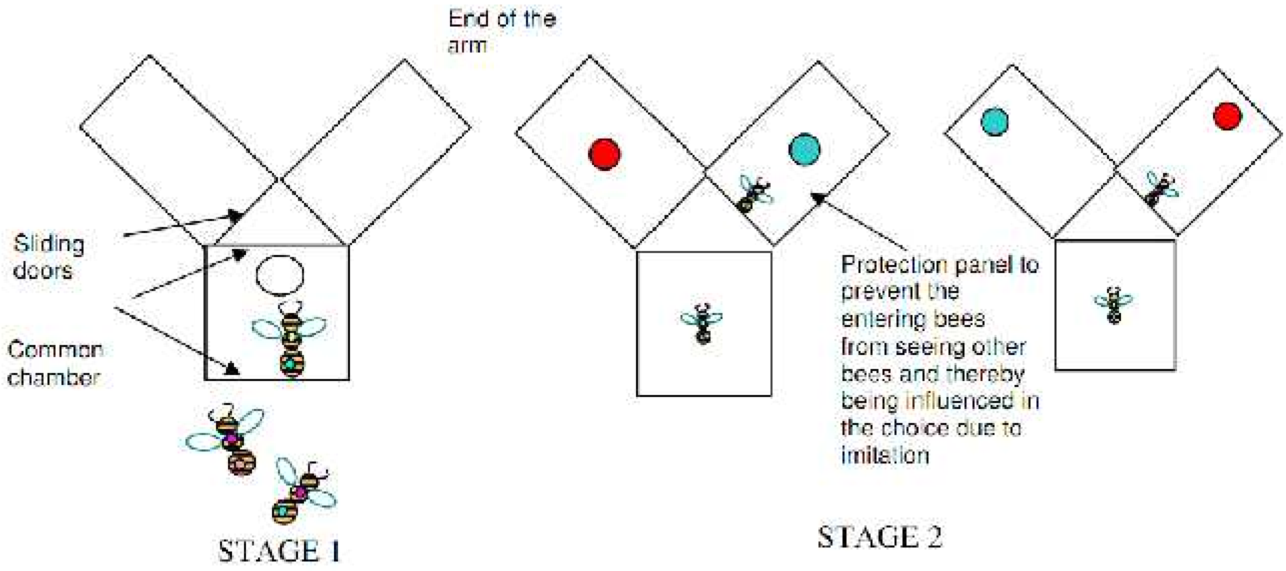


Diagram of the training procedure designed to associate the reward with the blue colour, namely with the visual stimulus chosen to train the bees to achieve correct orientation inside the Y labirinth. Bees were marked with colours on the thorax and the abdomen.

The bees had no difficulty in associating the colour with the reward, as this is a task known to be very simple for bees. Thus all the bees had learned how to follow the transfer of the correct dispenser from one arm of the labyrinth to the other. However, the data indicate that bees contaminated with thiamethoxam dust experienced considerable difficulty in recovering the correct memory of the colour associated with the reward. **Correct choice percentages were below 50%**. This result, although preliminary, given the low number of bees submitted to the test, **would appear to suggest that individuals treated with thiamethoxam recover memory of the wrong colour at the moment of making their choice**.

6.4 Effects of ingestion of nanodoses of clothianidin and fipronil on bee homing ability and on forager behaviour in relation to the hive

The experiment was conducted using clothianidin, to complete the trial begun in 2010 in which two groups of bees were subjected to ingestion treatment involving, respectively, 0.7 and 0.47 ng/bee of clothianidin. The 2010 experiment had shown marked impairment both of ability to return to the nest and also of foraging frequency after only 1 ingestion of the above cited doses.

Due to the restricted time available for the project, it was not possible to complete all the protocols. Protocols 1 and 2 have been completed. Examination of the videorecordings is in progress.

The observations conducted at the moment of release after treatment and in the nest showed **normal behaviour for the untreated bees** (direct flight towards the nest, discharge of nectar, interactions and exchange of food with the companion bees, flying out and immediate return to the dispenser for a renewed collection).

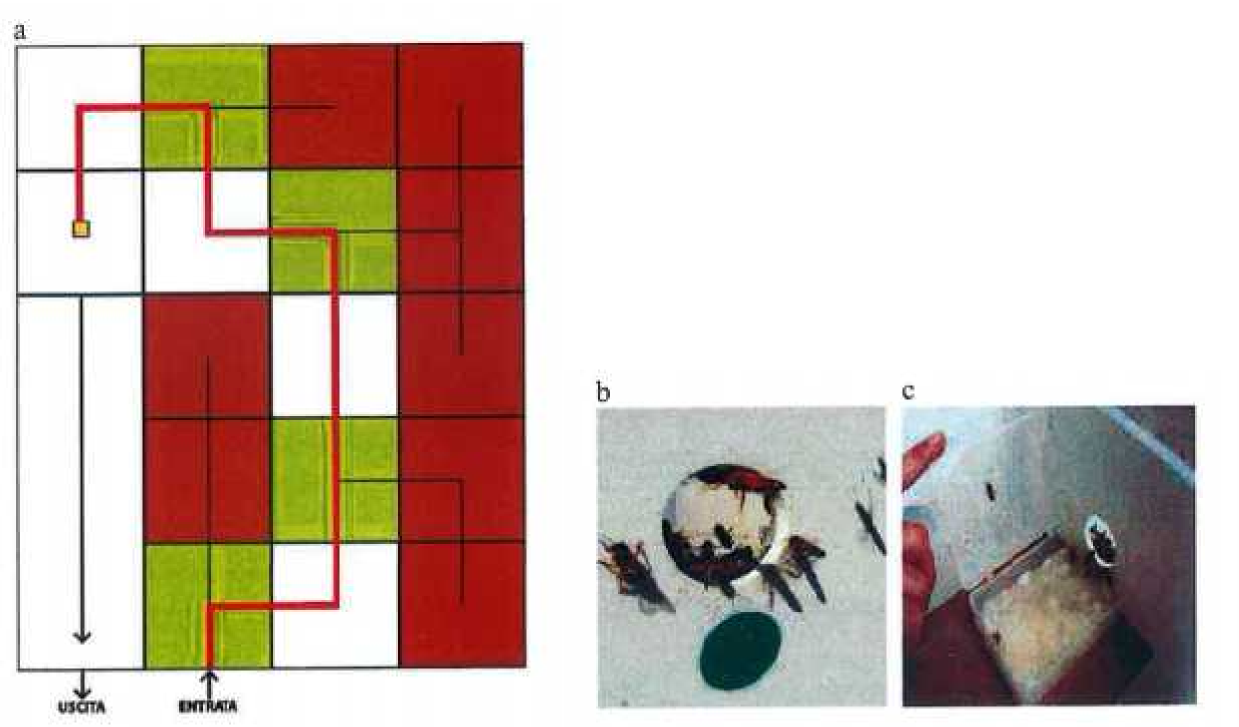
Thus treatment even at these extremely low doses had a marked impact on bee presence at the feeding point 24 hours after treatment: whereas 86.7% of control bees were present at the dispenser, this percentage decreased to 16.7% in treated bees.

The interpretation is that bees which overcame the poisoning phase were able to resume foraging with a frequency similar to that of the non-treated controls; however, half of the treated bees were not present at the dispenser at 24 h after treatment.

6.5 Bee disorientation tests in the complex labyrinth

The experiment consisted in training the bees to move along a route inside a labyrinth in order to gain access to the reward in the form of sugar syrup. A bee's orientation within a complex labyrinth is based on associative learning that links a visual mark with the reward that consists of sugar syrup. The aim of the test was to assess the sublethal effect of clothianidin, an active ingredient used in maize seed dressing, on bee orientation ability within the labyrinth. **Together with the PER (Proboscis Extension Reflex) test, this method could be used in future as part of official guidelines (EPPO, OECD) to assay the sublethal effects of pesticides. However, unlike the PER test, on which many publications are available and which has a methodology that is by now fairly standardised, the complex labyrinth test has so far been little used.**

Therefore the second aim of our experiment was **to set up a protocol using the complex labyrinth to assay the sublethal effects** of pesticides on bees.



Example of a route within the labyrinth (a) with the 5 "non-decision box" (in light green), along which the bee has to move, throughout holes marked with a green dot (b), in order to reach the dispensed (small yellow square) (c).

6.6 Conclusions

Olfactory memory

The experiments confirmed the results of the experiments conducted on dust-derived contamination in 2009. **All the active ingredients assayed, even at these extremely low concentrations, were found to be able to impair olfactory learning and memory.**

Colour recognition and orientation in the Y labyrinth

The experiments were conducted to assess **the orientation ability** of unconfined bees in a simple Y labyrinth. Results showed that the **sub-lethal doses of thiamethoxam contained in dust deposited at 5 m by the modified seeding machine** (5 % of active ingredient as compared to the unmodified seeder) **were capable, after 24 hours, of impairing the bees' ability to move towards a known food source** (only 50% of bees returned to the labyrinth after 24 hours) and to recognise the colours associated with the sugar reward. We even recorded two cases of a bee repeatedly choosing the colour that had been associated with salt during the training.

Homing behaviour in relation to the nest

Research on the effects of sub lethal doses on homing ability and on bee behaviour in the hive is in progress. However, our **preliminary results already provide clear evidence that even at extremely low doses,** such as that applied in protocol 1 (0.092 ng/bee, equivalent to 1/10 of the dose used in the PER experiments concerning the effects of ingestion contamination on olfactory memory, 2009 and 2010), **contact with the active ingredient by ingestion led, after a number of ingestions that varied from 3 to 10, to poisoning of the bee with impairment in foraging activity and loss of orientation ability, as tested after 24 hours**.

The number of repeated occurrences of micro-intake of active ingredient necessary to induce the blocking of bee activity was variable, with some bees failing to return to the feeding point after 3-5 administrations, and simply stopped at the nest, while others performed 10-12 flights (which effectively meant exposure to 10-12 administrations). Only one bee was present in the nest after 24 hours, whereas none of the control bees were missing.

Results obtained in the two years trials were similar and showed that bees submitted to a single administration of a quantity of clothianidin defined as 0.47/bee showed marked immediate effects: the bees returned to the nest but remained immobile and did not exchange food or discharge the honey sac. Exposure to clothianidin significantly reduced foraging flight frequency during the hours immediately after ingestion, and on the following day the foraging frequency of many bees was nil.

It is evident that clothianidin, administered according to various protocols that simulated different modes of contact or ingestion in the field, produced in all cases a reduction in foraging frequency, impairment in behaviour of bees that returned to the nest, or loss of orientation with failure to return to the nest when the dose reached roughly 0.4 ng/bee through multiple exposures to very low doses.

Therefore, it can be concluded that exposure to extremely low doses of neonicotinoids and fipronil, either in the form of contact from dust or intake by ingestion (from nectar, pollen or contaminated

water) impairs a bee's ability to adopt the appropriate behaviour required for fulfilling its functions. If exposure is repeated (as is obviously the case if the dose is not such as to kill the bee immediately), this leads to disorientation (inability to return to the nest) and/or death.

The logical conclusion to be drawn is that since the bees forage en masse on the same crops, **if there is an outbreak of contamination among a significant number of foraging bees then this impairs the equilibrium of the entire colony. The assumption of sub lethal doses of these active ingredients can therefore contribute to creating a state of "chronic weakness" of the colony.**

Disorientation trial in the complex labyrinth

One of the aims of this experiment was to set up a protocol for evaluating the effects of neonicotinoids on bee orientation ability in a complex labyrinth, which we adapted to our experimental conditions. Our study was initially based on the research by **Decourtye et al. (2009),** who pioneered the first experiments in a tunnel were carried out, but our environmental conditions proved **unsuitable for training a large number of bees to move along the route in the labyrinth.** Furthermore, Decourtye and co-workers divided the experimental procedure into three phases (two "untreated" periods separated by a "treated" interval), in which the bee response was evaluated before and after exposure to the pesticide. **In our view, a possible consequence of this method is that the experiment may be subject to the "time" variable. Thus one of the aims of our study was to assay both the active ingredient-treated and the control bees at the same time.**

In all the trials (with the exception of trial 2), **no significant differences** in the percentages of bees that returned and in the time taken to return and to move along the route in the labyrinth were observed **between the controls and the active-ingredient-treated bees**. The quantities of active ingredient assayed in our study, administered to the bee as a single dose and not ad libitum, are comparable to the active ingredient concentration theoretically observable on drops of dew or nectar on the surrounding vegetation, calculated as roughly 15 μ g/L (based on the assumption that a dewdrop amounts to 0.05 μ L).

The effects of these quantities of active ingredient, administered as a single dose, are less evident and less clearly noticeable in terms of immediate effect or effect a few hours after exposure, as compared to administration ad libitum (the latter, however, being the more likely case in nature). Other studies have shown that sublethal effects appear after 3 hours (Bortolotti et al., 2003; Medrzycki et al., 2003), but with higher doses. This notwithstanding, certain consequences were detected even in our extremely low dose study, as pointed out in trial 2, where a significant number of active ingredient-treated bees failed to return to the labyrinth.

7. Possibility of adopting integrated control for virus control in maize crops

Main aim: assessment of the possibility of integrated control against viruses in maize crops and identifying the possibility of control with reduced application of insecticides.

Intermediate aim: assessment of the incidence of viruses, hybrid susceptibility, possibility of vector monitoring and of post-emergence treatment on vectors.

It is known that Rough Dwarf Virus is more widespread in certain types of geographic areas with specific characteristics (high plateau with a marked incidence of grassland, including stable grassland, and uncultivated areas of Friuli, Veneto and Piedmont). The experimental sites were selected from areas where the presence of plants affected by rough dwarf disease had already been ascertained in the past or from zones having numerous uncultivated areas surrounding the planted plots. Due to the marked presence of grassy areas, at times partially forested or intermixed with woodlands, in the vicinity of the experimental plots, 7 out of the 11 selected sites can be considered to have a potential for elevated virus vector presence. The other 4 fields can be considered to have medium or medium-low pressure, linked only to the grassy areas round the edges.

At each site, in order to distinguish the virosis factor from pressure exerted on the crop by other pests, attempts were made to select plots with low populations of western corn rootworm nd wireworms.

The crop management technique, with conventional ploughing and complementary tillage, was homogeneous in the trial plots. In all plots, treatment against pyralidae was applied between 10 and 25 July in order isolate the main factor under study (virosis) more effectively and to reduce the possible incidence of pyralidae on within-field variability.

Incidence of plants with rough dwarf symptoms was found to be very low. Presence of symptoms was detected only in 2 plots out of 11 and was **much lower than 1% of plants observed,** despite the choice of plots with special risk factors for this disease. Incidence was found to be significant, albeit still contained, only in one site, where the presence of the virus disease had also been ascertained analytically in 2010**.**

Post emergence insecticide treatment showed lower levels of damage compared to the control, but damage was never statistically significant

7.3 Conclusions

Although the trials were limited to only one growth season, the data obtained appear particularly interesting with regard to three aspects:

1) **virus incidence seems low and limited to specific geographic areas** already found in the past to have significant disease presence;

2) **clothianidin used as a dressing on sensitive hybrids succeeds in significantly reducing the incidence of rough dwarf disease even in sensitive hybrids;**

3) **a similar reduction in disease incidence can be achieved by adopting resistant hybrids without using insecticides.** The presence of virus vector species (Delphacidae) and, in particular, presence of the vector of rough dwarf disease (L. striatellus), is ubiquitous. The latter species was identified in all the grassy areas bordering on the trial plots. However, the species appears to have a low tendency to enter into the plots, as few specimens were captured inside the plots.

A scanty, statistically non significant, effect of the insecticide dressing on productions of grain maize was confirmed.

8. Synergistic interactions between stress agents and bee colony collapse

8.1 Introduction

A number of authors have hypothesised that the recent phenomena of bee die-off also reported in Italy may derive from interactions between pathogens and other stress factors, such as parasites.

Overall, these results suggest that Varroa exerts a synergic effect with regard to DWV, in such a manner that its influence on bee immune defences leads to a transition from innocuous latent infections to disastrous viral explosions.

The sensitivity of the immune response factors, identified by the present study, to various environmental stress factors suggests that not only disease but also forms of stress deriving from nutritional deficiencies or from sublethal doses of pesticides can interfere with defences against pathogenic organisms, and can thus have harmful effects on bee health (Desneaux et al., 2007; Mullin et al., 2010).

The trials described here aimed to evaluate, in controlled laboratory conditions, the impact of the neonicotinoid pesticide clothianidin on DWV and on survival of bees exposed to doses up to 15 times lower than the officially reported LD50 values.

The data obtained indicate that in our trial conditions clothianidin was capable of inducing proliferation of latent viruses, and this proliferation was associated with mortality rates that were higher than expected.

Since the antiviral immune response, similarly to numerous reactions to biotic and abiotic stress agents, is also modulated by the Toll pathway (Zambon et al., 2005; Sabin et al., 2010), this study aimed to assess the impact of clothianidin on the Toll pathway, using transgenic lines of *Drosophila melanogaster*.

The results obtained suggest that the neonicotinoid under examination has a negative influence on this transcriptional activation of antimicrobial molecules.

8.5 Conclusions

The obtained results indicate that clothianidin is able to promote DWV proliferation. Although DWV is commonly present in bees as a latent infection, its proliferation has very evident negative consequences for bee survival. The adverse effect of clothianidin on modulation of the Toll pathway–mediated immune response could explain the proliferation of latent DWV observed in bees exposed to this pesticide. The extent of the negative impact deriving from this increment, which in turn is due to the viral load, depends on the state of infection at the moment of exposure to the active ingredient and also on other concomitant stress conditions (parasites, diet, etc.), which influence the level of immune defences. This may also provide a partial explanation of the variability of toxicological data recorded in the various studies performed. **Therefore, in evaluating the effects of clothianidin and other pesticides on bees, it is important to consider this additional aspect of indirect toxicity, the final outcome of which can vary as a function of colony health.**

Scientists and Institutions in charge of the trials

1. Evaluation of the productive and agronomic utility of maize seed treatment and persistence in plant tissues of the active ingredients used for seed coating

Dr. Mario Motto - Director of Agricultural Research Council- Maize Research Unit (CRA - Unità di ricerca per la maiscoltura), Bergamo, Italy; Dr. Carlotta Balconi - Agricultural Research Council- Maize Research Unit (CRA - Unità di ricerca per la maiscoltura), Bergamo, Italy; Dr. Lorenzo Furlan - Veneto Agricoltura

2. Effects induced in bees by contact with dust during flight over a field sown with coated maize seed

Prof. Vincenzo Girolami - Department of Environmental Agronomy and Plant Production-Entomology (Dipartimento di Agronomia Ambientale e Produzioni vegetali – Entomologia), University of Padua, Italy

3. PER *(Proboscis Extension Reflex)* test used to evaluate the effects of clothianidin, imidacloprid, thiamethoxam and fipronil administered as contaminated abrasion-dust

Prof. Stefano Maini - Department of Agroenvironmental Sciences and Technologies (Dipartimento di Scienze e Tecnologie Agroambientali), University of Bologna, Italy; Dr. Claudio Porrini - Department of Agroenvironmental Sciences and Technologies (Dipartimento di Scienze e Tecnologie Agroambientali), University of Bologna, Italy; Dr. Bettina Maccagnani - Agriculture and Environment Research Centre "Giorgio Nicoli" (Centro Agricoltura Ambiente "Giorgio Nicoli"), Crevalcore (BO), Italy

4. The monitoring network

Dr. Franco Mutinelli - Animal Disease Prevention Institute of North-East Italy (Istituto Zooprofilattico Sperimentale delle Venezie), Legnaro, Italy; Dr. Claudio Porrini - Department of Agroenvironmental Sciences and Technologies (Dipartimento di Scienze e Tecnologie Agroambientali), University of Bologna, Italy

5. Determination of the minimum level of dust dispersal during coated maize seed sowing with modified seeders and estimated effects on bees

Dr. Daniele Pochi - Agricultural Research Council- Agricultural Engineering Research Unit (CRA - Unità di ricerca per l'ingegneria agraria), Monterotondo (Rome), Italy; Prof. Vincenzo Girolami - Department of Environmental Agronomy and Plant Production-Entomology (Dipartimento di Agronomia Ambientale e Produzioni vegetali – Entomologia), University of Padua, Italy; Dr. Marcello Biocca - Agricultural Research Council- Agricultural Engineering Research Unit (CRA - Unità di ricerca per l'ingegneria agraria), Monterotondo (Rome), Italy; Dr. Elisa Conte - Agricultural Research Council- Plant Pathology Research Centre (CRA - Centro di Ricerca per la Patologia Vegetale), Rome, Italy; Dr. Patrizio Pulcini - Agricultural Research Council- Plant Pathology Research Centre (CRA - Centro di Ricerca per la Patologia Vegetale), Rome, Italy

Other collaborators: Dr. Roberto Fanigliulo, Dr. Marco Fedrizzi (CRA-ING); Dr. Lucia Donnarumma (CRA-PAV), Dr. Matteo Marzaro; Dr. Linda Vivian Department of Environmental Agronomy and Plant Production-Entomology (Dipartimento di Agronomia Ambientale e Produzioni vegetali – Entomologia), University of Padua, Italy

6. Sub-lethal effects of neonicotinoids and fipronil on learning and memory of odours and spatial orientation

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7. Possibility of adopting integrated control for virus control in maize crops

Dr. Lorenzo Furlan - Veneto Agricoltura

8. Synergistic interactions between stress agents and bee colony collapse

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