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The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators

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Abstract

The monitoring of pesticides with honey bees, which has been carried out by our group since the beginning of the 1980's, is an extremely important technique not only for proving potential bee poisoning risks by the use of pesticides, but also for determining the degree of environmental contamination due to plant protection products. In fact, because of its morphological and ethological features (such as its wide area of patrol and its intense foraging activity), the honey bee can be considered an excellent bioindicator. In many cases, pollution caused by abuse or by erroneous application of pesticides could not be proven without the help of honey bees.

In this research work, which is being applied hitherto in some areas surrounding Bologna, each monitoring station consists of two beehives equipped with collection cages for dead bees. Once a week, families are checked and the number of dead bees is recorded. When the mortality rate exceeds the critical threshold (250 bees/week/station), laboratory analyses are carried out. Monitoring techniques, chemical and palinological analyses, and data processing (through the Environmental Hazard Index) enable us to characterise areas, to indicate periods of major bee poisoning risk, and to identify the most frequently used pesticides (also those that are prohibited) and the crops treated.

Our studies with honey bees reveal the type of plant protection management applied to the area under investigation and allow us to prove the application of molecules not permitted under certain circumstances or even forbidden.

Key words: monitoring, *Apis mellifera*, environmental hazard.

Introduction

For about twenty years our research group at the "Guido Grandi" Institute of Entomology of the University of Bologna has been studying the relationship between honey bees and the pesticides spread throughout the agro-ecosystem.

The systems for monitoring honey bee poisoning incidents with pesticides can be of various kinds. We can describe six levels, based on different degrees of complexity and sensitivity, and depending on the context and the objectives pursued (table 1).

In the first level the context is "apiculture", and the beekeepers communicate general poisoning incidents to the authority. In the other levels, the context is "monitoring" or "pollination and monitoring", and more specific information, also about the causal agent, is provided.

The higher the level, the more specific and expensive are the techniques. In the first two levels, traditional hives equipped with dead bee traps are used, whereas in the higher levels also the use of pollen traps and the collection of forager bees are involved. In the two highest levels, also the family strength is evaluated; only in the highest level the number of foragers bees is evaluated with electronic bee counters.

The higher the level, the more time commitment, professional qualification of the operators and costs increase.

The monitoring level we are applying in our monitoring campaign is between number three and number four, representing a good compromise between costs and achieved information.

Since 1980, this strategy has been applied in 34 provinces, townships or inter-municipal territories through much of northern Italy. Overall, 400 monitoring stations have been installed to cover a total territory of 2800 km² (the Italian territory is 301308 km^2). Between 1983 and 1986 in particular, the analysis of 581 gathered dead bee samples revealed which compounds were most widely used in that period in cultivated fields, above all in northern Italy (Celli *et al*., 1988a, 1988b).

In those years, bee poisoning incidents were caused primarily by treatments carried out in orchards, vineyards, on seed crops and by pollution due to drift on spontaneous plants. The most frequently found pesticides were phosphorganics (dimethoate, parathion, azinphos-methyl, methyl-parathion, omethoate and metamidophos), followed by carbamates (carbaryl) and clororganics (endosulfan). Dithiocarbamates were almost always found in the dead bees samples, together with other products, insecticides for the most, which were the ones to blame for the bees' death. These include dimethoate, a compound serving a huge variety of purposes but often misused. The use of parathion, a compound that has prevailed on the agricultural scene for years, is instead indicative of backward farming techniques and disregard for the environment. In fact, this compound is deadly for a large number of beneficial organisms.

Table 1. Different levels of environmental monitoring with honey bees (Accorti 1994, modified).

Level	I	II	III	IV	V	VI
Context	Apiculture	Pollination	Pollination & monitoring	Monitoring	Monitoring	Monitoring in proportion to agent
Count method	manual	manual	manual	manual	manual	automatic
Management	traditional	traditional	specific	specific	specific	specific
Equipment and techniques[1]	a, c	a, c	a, c, d	a, c, d, e	a, c, d, e, f	b, c, d, e, f, g
Frequency of sample-gathering	1 x 7 days	1-2 x 7 days	1-3 x 7 days	1-5 x 7 days	3-5 x 7 days	continuous
Time commitment	+	+/++	+/++	+++	+++	+/++++
Professional qualification of operator	+	+	+	++	+++/++++	++++
Sensitivity	+	+/++	++/+++	++/+++	++++	++++
Costs	+	+	++	++	+++	++++
Field applicability	++++	+++	+++	++	+	+

[1] a=traditional hive; b=specific hive; c=dead bee trap; d=pollen trap; e=collection of foragers; f=family strength; g=electronic bee counter.

In recent years more specific and extended studies have been conducted in many areas of Italy, especially in the North (figure 1).

The pesticide monitoring strategy was first successfully applied in the province of Forlì (figure 1), an area of intensive orchard cultivation; thanks to the financial support of the Provincial Authority, it was possible to pursue the investigation uninterruptedly from 1982 to 1993.

The data gathered in this period brought to light a decisive trend of improvement as far as the pesticide contamination of the agro-ecosystem of Forlì is concerned. After various training sessions with the growers, in fact, bee poisoning incidents decreased from a mean of 8-9 per year in the eighties to 3 per year in the nineties.

This was ascribed above all to a new awareness among farmers, who were more careful about using pesticides properly and choosing plant protection products that did not threat the environment and, in particular, were not harmful to beneficial insect species (Celli *et al*., 1987; Porrini, 1991, 1996).

Materials and methods

Monitoring protocol

According to the protocol we adopted for our monitoring system (table 2), two hives per station are used. The hives must be homogenous (in terms of family strength) between them and with those of the other stations, and they are constantly checked for sanitary purposes. Each hive is equipped with a collection cage for dead bees; the trap we actually use for dead bee collection is the type "underbasket" (Accorti *et al*, 1991); as shown in past researches, among those available "underbasket" traps are the most suitable in retaining dead bees. Once a week, families are checked and the number of dead bees is recorded. When the mortality rate exceeds the critical threshold (250 bees per week per station, Porrini *et al.*, 2002), laboratory analyses are carried out.

Chemical and palinological analyses are performed. With the first one we detect the pesticides present in the dead bee sample, which are probably responsible for the bee poisoning. With the latter we identify the kind of pollen found on the dead bees' surfaces. This analysis, together with the crop-growing maps of the area surrounding the monitoring station, enables us to make hypothesis on how and where the bees got intoxicated.

In several cases, palinological analysis helped us to identify not only which crop was treated and in which area, but also the causes of the bee poisoning. In other cases we had to confine ourselves to suppositions for different reasons (low number of pollen grains on dead bee bodies, no specific botanical identification, drift of spontaneous plants all over the territory, which can not be reported on the map, etc.).

Table 2. Monitoring protocol adopted for pesticides

Hives per station	two
Used matrixes	dead bees
Dead bee collection traps	"underbasket"
Frequency of sample gathering	weekly
Critical mortality threshold	250 dead bees/week/station
Analyses	chemical and palynological
Other tools	crop-growing maps

Figure 1. Areas where our pesticide monitoring campaigns with honey bees have been carried out.

Index of Environmental Hazard

Maps that plot the level of environmental hazard in the territory under investigation are updated monthly. These maps are defined by a two-way index (IEH – Index of Environmental Hazard, table 3), which appropriately establishes the degree of pollution by pesticides. This index is obtained by intersecting the mortality class of one station (monthly mean of the number of dead bees per week) with the IPT (Index of Pesticide Toxicity) of the pesticides found on the dead bees at the stations. The IPT is an index that takes into consideration the toxicity and the persistence of the pesticide, and is calculated as follows:

$$(IPT) = f_{corr} \sum_{c=1}^{N} \frac{(ct)_c (fp)_c}{N}$$

Where:

$(ct)_c$: compound toxicity class with respect to bees, normalized to the highest value;

$(fp)_c$: compound persistence factor, normalized to the highest value;

f_{corr}: correction factor. This factor must be used only if, in the same month, some of the chemically analysed honey bee samples that exceeded the critical threshold of mortality were positive while others were negative; the purpose of the correction factor is to give a "weight" to the latter samples in the formula. It is calculated as the ratio between the mean number of dead bees corresponding to negative samples and the overall mean number in the period taken into account. Only values greater or equal to 1 are considered;

N: number of positive bee samples;

Note: when several pesticide residues are found in a single bee sample, the numerator in the formula for that sample can be obtained by means of a suitable averaging procedure.

Results and discussion

Our monitoring campaigns carried out in several areas of Italy (figure 1), provided helpful information about the plant protection management practices used, because they enabled us to identify the active ingredients most frequently applied in each area (figure 2).

With the monitoring system we can detect cases of abuse, misuse or forbidden use of pesticides (Porrini, 2002). Here we report some examples of these three cases.

An example of abuse of pesticides is shown in figure 3: dimethoate is widely used in many cereal growing areas to control aphids on wheat around mid May; however, its application is often useless, since the damage caused by the phytophagous insects is often lower than the cost of the treatment itself. Dimethoate not only harms the bees and the pockets of the growers, it also impoverishes the beneficial entomofauna (entomophagous ladybirds) within the agroecosystems.

Table 3. Index of Environmental Hazard (IEH). A_1: persistent, A_2: worrying, A_3: substantial, A_4: considerable, B_1: elevated, B_2: important, B_3: widespread, C_1: medium average, C_2: medium-low, C_3: moderate, D_1: low, D_2: limited, D_3: minimal, D_4: absent.

IEH	Mortality classes (monthly mean of the number of dead bees/week.)			
	0 - 200	200 - 400	400 - 800	> 800
Residue-free samples or mortality below critical threshold	D_4	D_2	C_3	C_1
0 < IPT < 0.125	D_3	D_1	C_2	B_3
0.125 < IPT < 0.25	D_2	C_3	C_1	B_2
0.25 < IPT < 0.375	D_1	C_2	B_3	B_1
0.375 < IPT < 0.5	C_3	C_1	B_2	A_4
0.5 < IPT < 0.625	C_2	B_3	B_1	A_3
0.625 < IPT < 0.75	C_1	B_2	A_4	A_2
0.75 < IPT < 0.875	B_3	B_1	A_3	A_1
IPT > 0.875	B_2	A_4	A_2	A_1

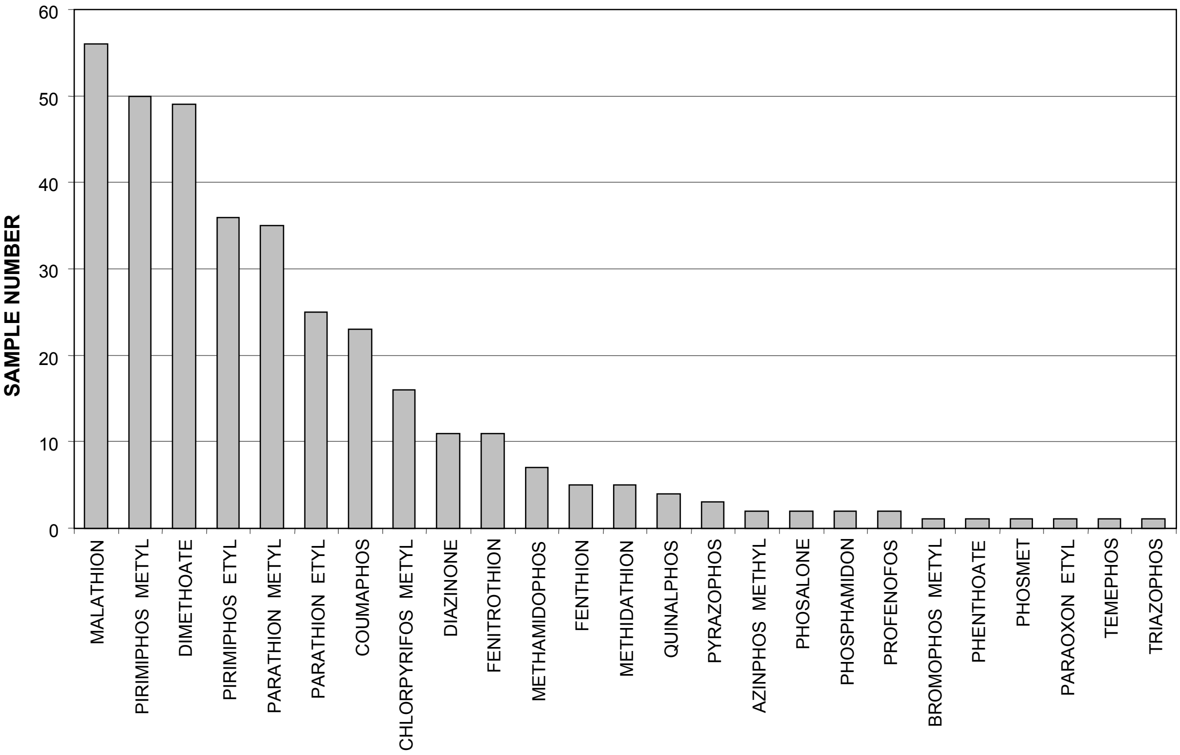

Figure 2. Frequency of active ingredients found in 105 samples of dead honey bees coming from Granarolo (Bologna) and Ozzano (Bologna) in 2000.

In several monitoring campaigns, in dead bee samples we found obsolete active ingredients (such as parathion, methamidophos or methidathion), dangerous to both the environment and humans. These plant protection products indicate not specialised agricultural practices, which, above all, do not respect the territory. The decision to apply these products seems to be dictated more by commercial motivations than by considerations regarding plant protection. In fact, very often the dead bees containing residuals of these active ingredients arrive from areas where agriculture is not specialised but where a type of "part-time" conduction occurs, or from private gardens, generally located in the immediate surroundings of the cities. Usually, these treatments are conducted by people not informed about the application techniques of pesticides.

Monitoring with bees allows us also to evidence the application of molecules, not permitted under certain circumstances or even forbidden. In 1995, for example, in one of our monitoring campaigns, lindane was found in dead bee samples from hives placed in the city centre of Ravenna, even though no conditions would have justified its use. In fact, in Italy, the application of this product is permitted only in limited agricultural ambits, such as soil disinfestation in sugar beet cultivations, as a disinfestant on stored cereals, and in seed tanning.

Another case occurred in Ozzano (Bologna), (figure 4). In June 1998, fenoxycarb was detected in a sample of dead bees. The use of this product, an IGR (Insect Growth Regulator) chemically classified as a carbamate, is recommended primarily on apple, peach (mining and codling insects) and grapes (moths), but its sale and application are forbidden all over Italy, because of its harmful effects mainly on the silk worm. Nevertheless, fenoxycarb was applied, probably against the vine moths (*Lobesia botrana* and *Eupoecilia ambiguella*).

Conclusions

In each of the cases reported above the improper use of pesticides was noted only because of the presence of the bees. Pollution by pesticides can be detected promptly and continuously at low costs by using bees as bioindicators, since they are able to signal immediately and unambiguously the incorrect use of pesticides. Anyway, some of the new insecticides do not induce high bee mortality but, even at low doses, they may cause severe behavioural changes, which could damage the entire family. They are also difficult to detect with chemical analysis and thus our tools (mortality and residues) are not always able to reveal them. Therefore we are now introducing some changes in our protocol, in relation to these new effects.

In Italy, right now, a national monitoring network of bee poisoning incidents does not exist, even though the Italian Environmental Protection Agency (ANPA) is promoting its creation. We hope to find the necessary funds in a near future.

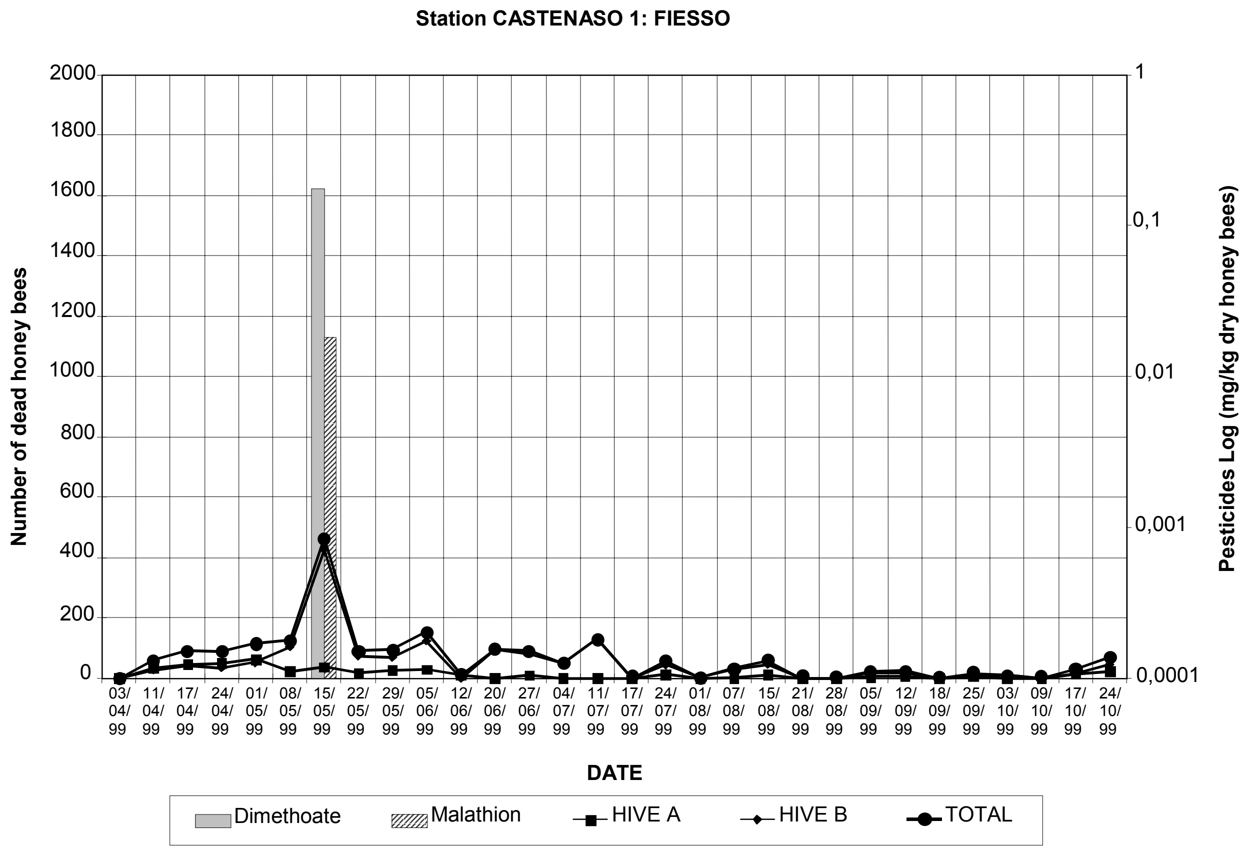

Figure 3. Example of abuse of pesticides (dimethoate against aphids on wheat).

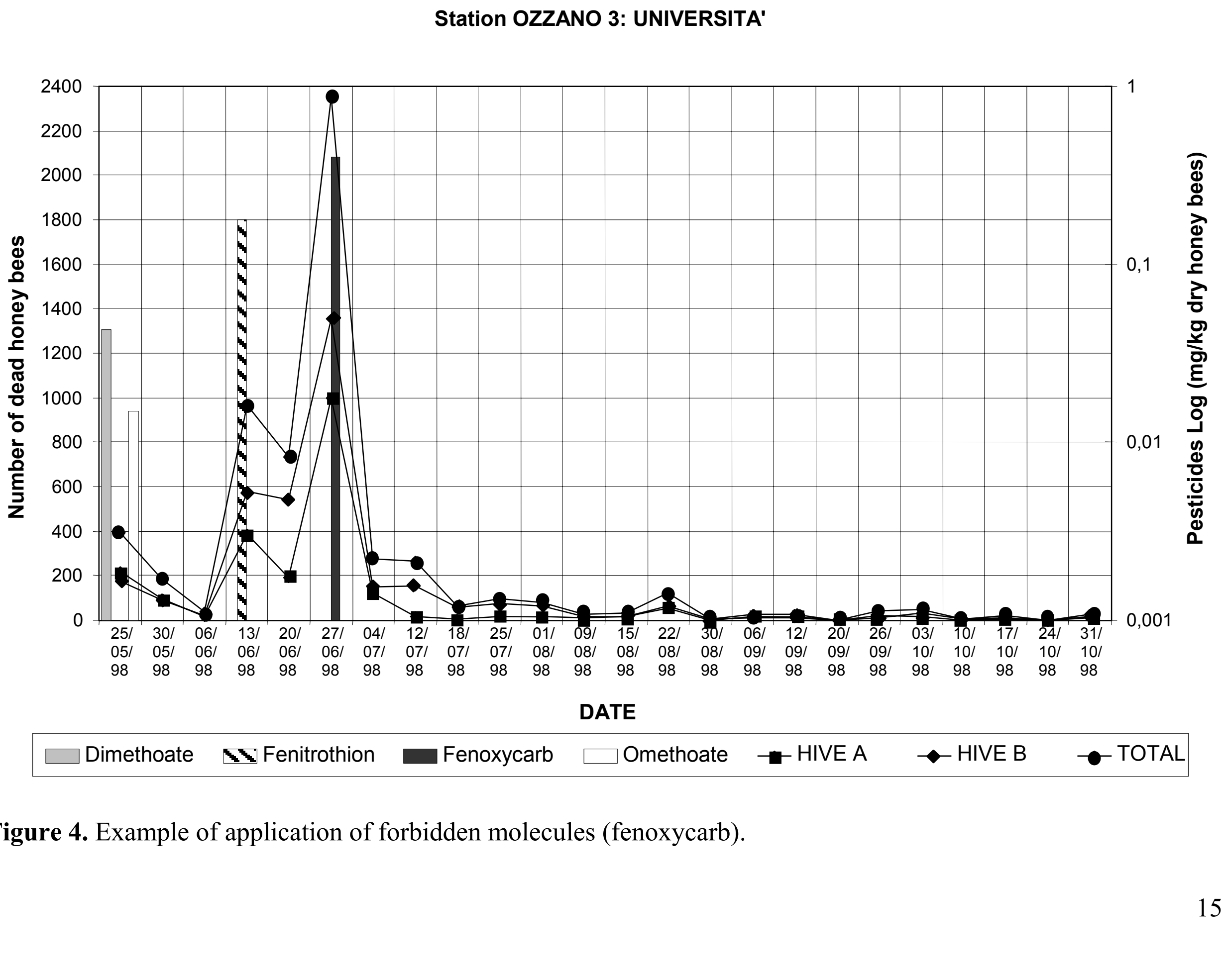

Figure 4. Example of application of forbidden molecules (fenoxycarb).

The new agriculture which we so much hope for, will have to strive for that the application of these molecules in our cultivated fields will decrease and, for this purpose, the honey bee can be of great help. In any case, let us remember that the honey bee is killed by pesticides when they are incorrectly diffused in the environment (cultivated fields or private gardens) either in a qualitatively or quantitatively wrong way, that is by not applying the recommended dosage or by not obeying the accepted technical norms of use (culling of spontaneous plants, absence of wind, absence of honeydew).

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