Bee gone: colony collapse disorder

For the past ten years there has been regular press documenting the collapse of bee hives around the world with a variety of theories put forward to explain it. ***Janine Kievits****, a beekeeper and member of a federation of environmental associations, assesses the evidence implicating the insecticides used to treat seeds.*

In 1993 beekeepers in southwest France raised the alarm for the first time: their bees, foraging on sunflowers, were affected by a mysterious disease which decimated the hives in days. The same thing was seen the following year. Very quickly they suspected Gaucho®, a new insecticide being used by sunflower growers. In contrast to other insecticides, systemic or contact, which are sprayed on the growing plant, this one is used to treat seeds. Treating the seed with Gaucho® is sufficient to protect the plant through its entire growing season. Although miraculous for the farmer, this quickly aroused the suspicion of beekeepers: if the active substance present in Gaucho®, imidacloprid, remains active long enough to protect plants throughout their growth, could it not be found in the flower parts visited by bees? And so, the controversy between beekeepers and pesticide companies started. Beekeeping is an important industry in France with about 84,000 beekeepers of which 2,900 are professionals. But agriculture is also important providing a key market for agrochemical companies. Each side in the conflict found allies, the beekeepers with civil society and the pesticide companies with seed producers and other actors in the agricultural world.

A global problem

At the same time, a similar problem was observed elsewhere. Spectacular losses were recorded in Italy, Spain, Greece, Belgium, UK and Germany. More recently the problem has been documented on the other side of the Atlantic. In the US, 30 to 60% losses have been sustained in California, with similar catastrophic losses reported in Texas and on the east coast; up to 24 states have reported the problem[1]. In Canada the situation is serious in several provinces[2]; the press in Quebec report 40% losses on average, with some beekeepers losing 85% of their bees. The Minister of Agriculture of New Brunswick has gone as far as to grant 100,000 canadian dollars (70,000 Euros) to beekeepers to restore their hives. Latin America has not been spared (Chile, Argentina, Uruguay), and news of similar problems in Burkina Faso and Taiwan is on the internet.

While there are no comprehensive statistics, the seriousness of this phenomenon is unquestionable. Some professionals have seen two thirds or three quarters of their livestock of hundreds of hives destroyed. And the problem does not just affect beekeepers but also farmers whose crops require pollination by nectar bees[3]. Not surprisingly almond and blueberry growers in the US and cranberry growers in Canada, are standing by the beekeepers to demand research into the causes and cures of this problem.

Symptoms

Under normal circumstances in winter a hive has several thousand bees increasing during the summer to several tens of thousands. But in hives affected by Colony Collapse Disorder (CCD) there may be only a few bees around the queen, or none at all. They are not seen leaving the hive, and where they go is a mystery.

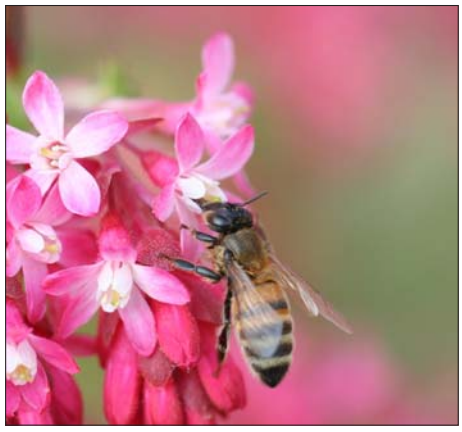
Although the problem was first noticed on sunflowers it quickly spread to areas where sunflowers are not cultivated. In these areas bees often forage for pollen on corn. Moreover, some flowering (and foraged) crops, such as white mustard, are sown in rotation after treated crops, such as winter barley. Beekeepers suspect these 'green manures' may be contaminated as the pesticides concerned are persistent in soils.

Nevertheless, the phenomenon of bee disappearances in different regions is not identical to that first observed in France. For example, in Belgium the bees do not disappear while their forage crop is in bloom as happens during a sunflower honeyflow, but at the end of autumn or at the beginning of spring.

Moreover, even on sunflowers different phenomena are seen. For example, sometimes the bees do not disappear, but shiver and cluster in heaps on the flowers or next to the hive. They stop foraging and the hive only produces a small fraction of the typical 60 to 80 kilogrammes of honey, or even completely collapses. In some cases significant changes in behaviour are seen. Some hives swarm before building royal cells, a significant number of fertilizations fail, some swarms leave without their queen, and young queens are replaced by bees, and so forth. Could such varied phenomena be ascribed to only one cause?

A social insect

To decipher the cause(s) of these phenomena needs a minimal knowledge of bees, their behaviour and diseases. Bees live in colonies whose numbers vary according to season. The queen starts laying eggs at the beginning of spring, before foraging flights start. The first larvae are fed with the reserve of pollen from



Foraging bees *Photo: CARI*

the previous summer which has been transformed into a mush by the nurse bees (pollen is fermented before use so that it can be digested by the larvae). The colony gradually develops, the queen laying more eggs as young bees are born that will be able to nurse new larvae. When fully developed, the hive contains about 50,000 to 60,000 bees of which 10,000 are forager bees that will gather enough nectar to form honey reserves for the hive over winter. At least 20 or 30 kilogrammes of honey are made from 50 to 100 kilogrammes of nectar carried by bees in loads of 50 to 70 milligrammes at a time. To achieve this, foraging is coordinated. When a forager bee finds food she returns to the hive and dances on the comb. The precise movements of her dance communicate to the other bees the flight direction according to the sun, and the distance, to the source of nectar. The bees also exhibit other complex behaviours. They build combs with perfectly hexagonal and regular cells; nurse bee broods; keep the temperature and humidity of the hive right for the brood, and to preserve the pollen and honey; assess the qualities of a home for a new swarm; visually memorize the surroundings of the hive; and protect the hive against predators. All these skills are based on innate behaviours with some elements learned. And the survival of the hive depends on the integrity of these behaviour patterns. And given that such behaviour depends on the integrity of a nervous system where each synapse is important, the potential impact of insecticides, most of which are neurotoxic, can be appreciated.

Possible causes

A number of theories have been put forward to explain CCD. Could a disease be decimating hives? Could bees disappear simply due to lack of food? Is electromagnetic radiation from mobile phone masts confusing the bees? Or are bees being affected by pesticides sprayed on crops on which they are foraging?

Disease

As with any other species, bees are prone to disease. Currently, varroatosis is the most significant and is caused by a parasite, the varroa mite. This mite 'migrated' from an asian bee to the honey bee and while the asian bee has adapted to coexist with the varroa mite the

honey bee has not. So beekeepers have to treat hives to prevent varroa mite attack which can decimate them. Other diseases also affect bees. Nosema disease is due to a bacterium (*Nosema apis*), and the key symptom is diarrhea. Viruses also affect bees, inducing symptoms such as palsies or wing atrophy.

But clinical evidence supporting the idea that hives are collapsing due to disease is lacking. Varroa is clearly visible with the naked eye, and the level of infestation can be determined by counting the number of mites falling on the hive bottom board. Varroatosis hits and kills hives every year. But the proliferation of varroa in affected hives is clearly visible, and the hive does not disappear so quickly or in the same manner as those affected by CCD. In addition, no study has shown any correlation between the infestation level of an apiary and the disappearance recorded. And no link has been observed between the treatments applied to fight varroatosis and the level of losses in the apiaries.

Spanish researchers identified a new Nosema bacterium, *Nosema ceranae*, by DNA analysis, and put forward the idea that Nosema disease is responsible[4]. But bees in the affected apiaries do not have the typical diarrhea. Although one of the researchers postulated that this was a 'dry nosema disease' there is no proof that this Nosema is the pathogen related to CCD. Indeed as this new Nosema has only recently been identified it is possible that it has been present in European apiaries for decades without serious effect.

It also seems unlikely that the declining hives are affected by any known viruses. A study analysing the infectious characteristics of two of the more common pathogenic bees viruses examined their capacity to cause colony collapse[5]. The Acute Paralysis Virus seems to be too virulent (the infected bee dies before contaminating other hosts or vectors). The Deformed Wing Virus can cause a reduction in the number of overwintering bees and death of a whole colony as a consequence. However, the disease is visible in the hive - infected bees have atrophied wings and a small stomach. Also, infection by one of these viruses does not explain the rapid collapse of hives during sunflower harvesting.

CCD does not follow the pattern of an infectious disease. The problem generally concerns several neighbouring beekeepers simultaneously. One beekeeper with two apiaries may see one collapse while the other remains healthy; a beekeeper seeing the problem in one location may solve it by moving his hives to another place. The next year, other places will be affected. An apiary devastated one year may be unaffected the following year. If the problem was caused by a disease pathogen, beekeepers would transmit the pathogen between hives with their equipment and their gloves.

Lack of food

Wild bee populations shrink under the pressure of the agricultural practices that deprive them of the food sources they need. Although lack of food is a possible explanation it is unclear why this would only have started in the early 1990s. A reduction in the number and variety of food sources correlates with the evolution of farming that started in the middle of the 19th century[6]. The honey bee, with a foraging radius of up to three kilometers or more is less sensitive than wild bees whose foraging radius is much smaller, up to a few hundred metres. Also, the observed characteristics of the phenomonen do not match that of food deprivation. It is highly unlikely that a hive's food sources would change so dramatically within a two week period that a previously flourishing hive would decline so rapidly.

Electromagnetic radiation

Bees experimentally exposed to waves from a DECT site[7] become disorientated[8]. After eleven days of observation the weight and number of surfaces built within the irradiated hives were lower than in control hives. Those exposed to electromagnetic radiation have also lost forager bees. However, in reality bees are never subjected to waves of such intensity and there is no large-scale correlation between electromagnetic waves and bee mortalities. Indeed, hives in urban zones (with a higher density of mobile phone aerials) are usually healthy while hives affected by CCD are located in rural areas.

Pesticide exposure

The most likely explanation for CCD is exposure to one or more of four new pesticides sprayed on sunflowers and other crops on which the bees forage. Imidacloprid, thiamethoxam and clothianidine are neonicotinoids which are acetylcholine agonists. Fipronil, a phenylpyrazol, binds to GABA receptors. And so, all four perturb neuroreceptor function. The hypothesis that neurotoxic insecticides could perturb the highly complex behaviour patterns of the bee is highly plausible. During the honeyflow on sunflowers bees are directly exposed to nectar; at the end of autumn or during the first spring flights bees are exposed through the summer pollen consumed at the end of the season or stored for the winter.

The possibility of sublethal behavioural perturbation by neurotoxic insecticides has been tested by scientists, who analysed the behavioural effects of imidacloprid, fipronil and deltamethrin. They showed that these substances were present in the nectar and pollen of treated plants (something that companies initially denied but now admit[9]) and at very small doses can affect some reflexes of bees, their ability to learn and return to the hive after foraging[10]. This could then explain the disappearance of the bees.

France withdraws Gaucho®

These results were sufficiently convincing to lead to the success of an action by the French beekeepers to have Gaucho® temporarily withdrawn from use on sunflowers and corn in France.

The use of agricultural pesticides in Europe is being reviewed under EU Directive 91/414/EEC. In France and other EU member states, the competent Minister has to withdraw a pesticide's authorization if it does not fulfill certain health and environmental safety criteria. The beekeeping trade union, UNAF (Union nationale de l'apiculture française), has made strong claims that tests prescribed by EU Directive 91/414/EEC have not been carried out. When the risk coefficient (Hazard Quotient or HQ[11]) of a product rises above 50 it is compulsory to carry out a number of tests on bee larvae. For Gaucho®, used on corn, the HQs are 18,900 and 11,283 for oral and dermal contact respectively. Bayer Cropscience argues that the HQ value is irrelevant where seed treatments are concerned as the seeds are not consumed. However, the chemicals can be detected clearly in the nectar and pollen consumed by bees. And, of course, legally France's Council of State cannot skip a requirement of the Directive[12].

Only a few bees remain around the queen in hives affected by CCD *Photo: CARI*

In different judgements (the last one in 2006) France's Council of State declared that the conditions of authorization had not been fulfilled, compelling the Minister to suspend the use of Gaucho® until the EC completes its review of imidacloprid.

This has created a precedent for all EU Member States and has led beekeepers to put pressure on the European decision-making process over the four active substances. The Draft Assessment reports from the review process are available via the European Food Standards Authority (EFSA) website. These contain the same loopholes as those present in the French process: no tests have been carried out on larvae, and the high HQs are disregarded when the active substance is used as a seed treatment. And yet legally the assessment scheme must be respected or, if not relevant, it must be reviewed and revised.

Flaws in the process

Analysis of the reports shows that the current process is insufficient to credibly test the potential toxicity of insecticides used in seed treatments. The present scheme does not take into account the chronic toxicity of the prod-

ucts, despite the fact they are present in the plant throughout its period of blooming - around one month in the case of sunflower and rape. This situation is entirely new and does not occur even with spraying systemic insecticides (such as deltamethrin). In addition, only the lethal effects are taken into account. Sublethal effects which do not kill the insect, such as effects on behaviour, are not tested.

Other studies presented in the Draft Assessment reports are far from adequate. For example, in the studies on clothianidine, the egg laying capacity of the queen is assessed by a test on colonies of 500 bees. But a queen can lay up to 1,000 eggs per day and can continue laying until the colony is very large. So this test is entirely inadequate to test if egg-laying capacity is unimpaired. Another example comes from the fipronil report. This indicates that the figures reported for Toxicity Exposure Ratio are calculated based on the amount of nectar to which the bee is subjected. The amount considered in the file is 20µl (about 25 mg). However, in reality a bee consumes between 100 and 900 mg of nectar in five days depending on whether it is a hive bee or a forager bee[13]. A new calculation based on this higher figure shows a much higher Toxicity Exposure Ratio which could explain why hives collapse within four days on sunflowers coated with fipronil: after four days the bees have almost consumed the LD50. Another example concerns tunnel tests. The tunnels cover plots of treated seeds and are usually 10 metres long. At a distance of 10 metres the bee is guided by eyesight or even smell. And so, its ability to learn the direction to a food source and back to the hive, the mechanism believed to be affected, is never called upon. It is however exclusively these tests which are used when the reports conclude that the active substance has no effect on bee behaviour.

More generally, none of the tests presented prove that the bees have actually consumed the treated pollen rather than their stored pollen (in tunnel experiments the colonies used have food frames at the start and bees may be using this source of food). Moreover the tests on seed treatments lack positive controls because of the technical difficulty of defining such controls.

Considering these deficiencies, some twenty associations and beekeeping unions wrote to the Commissioners in charge requesting that the assessments be reviewed and the pesticides in question not added to Annex I until the requirements of the Directive are appropriately fulfilled.

However, the European Commission has a fixed timetable within which to review a substance. They would be unable to meet their deadlines if they started the review from scratch. Moreover, there are no bee experts in the Commission; they are in charge of the risk management and while EFSA is in charge of considering the scientific process of risk assessment.

So why did EFSA and Member State rapporteurs overlook these shortcomings? The answer probably lies in their lack of capacity given the magnitude of the task they are charged with. These bodies check that the required studies have been carried out using standard methods; but potential flaws in the process are not pointed out.

Two major failings

There are two major failings in the current review process. Firstly, it relies on studies supplied solely by the company putting the substance forward for review (the 'notifier', often the manufacturer). And no review of the scientific literature is required. For example, in the fipronil file, reports of the French Scientific and Technical Committee (CST), whose conclusions differ from those of the notifier (BASF), are not even mentioned[14]. Although France is the Member State reporting on this substance, and the present EFSA staff member in charge has seen the CST reports, no account has been taken of them.

The second major failing is that the whole assessment process takes place without any overview by civil society. Access to Draft Assessment files on the EFSA website is a welcome new feature, but the process does not require any stakeholder consultation. So there is no chance to counteract lobbying by the agrochemical companies.

Economics

A small number of substances generate a significant part of the sales of the agrochemical companies. Bayer Cropscience's 'Top 10' substances generate more than one third of their annual sales turnover. Two of these are implicated in the bee deaths: imidacloprid (587 million Euros) and clothianidine (110 million Euros), which is 12% of the company's sales (5,896 millions Euros). The cost to bring a product to market amounts to about 200 million Euros and so there is strong pressure on companies to ensure sales continue if possible. If a problem arises the public interest requires that a product already on the market be withdrawn from sale or its use limited to those considered essential (perhaps only to limit the appearance of resistance). However, this would drastically limit a company's market creating an acute conflict between public interest and the economic interest of the manufacturing company.

Prospects

In the US there are fewer wild pollinators than in Europe and farmers are more acutely dependent on the honey bee to pollinate their crops. So there is greater economic pressure to find real solutions to this problem in the US than there is in Europe. Will Europe take its fate into its own hands or wait for solutions to come from the US? If US investigations lead the Commission to withdraw substances from Annex I, this would challenge the authority of the EC to maintain the high level of environmental protection that it boasts. Let us hope that Europe will equip itself soon with a credible assessment process, based on independent expertise, and transparent processes. This would reassure its citizens of the competence and independence of the institutions supposed to protect their environment and their health.

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