THE SCIENCE OF BEE DEATHS

No buzz from the beehives

There is something seriously wrong with the honey bee: in the winter one third of the populations die off. Scientists are gradually gaining more insight into the possible causes of these bee deaths but cannot (yet) point the finger at one sole culprit.

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Bees have been failing to thrive all around the world for years now. Bee populations have been dwindling in Europe and North America since the 1970s. Their decline is not limited to the honeybee kept by people (*Apis mellifera*); wild bee species are having a hard time too. Bees and bumble bees account for an estimated 75 percent of the pollination in agriculture and horticulture. As a result there is talk of a looming pollination crisis that could have consequences for nature management and agricultural yields. It is not for nothing that there is growing international attention for the 'bee problem' and scientific research into the causes.

It is at the end of the winter particularly that beekeepers notice that the honeybee is not doing as well: more populations die off nowadays. The winter period is a hard time for bees: it is cold and there is a shortage of food. Of the 20 to 30 thousand bees inhabiting a hive in the summer, only about 10,000 are left at the end of the winter. Sometimes an entire population is wiped out. There have always been these wintertime deaths but the average death rate has risen dramatically in the past few years, sometimes by 30 percent. The population deaths can vary between countries and regions in the same year from 7 to 50 percent. The winter of 2012-2013 seems to have turned out not too badly in the Netherlands, says researcher Tjeerd Blacquière of Plant Research International, part of Wageningen UR. Surveys of beekeepers suggest a low death rate of between 8 and 15 percent..

PUBLIC DEBATE

A global search is going on into the mechanism underlying the bee deaths. And one of the possible causes, the neonicotinoids – a class of pesticides – has been an especial focus of scientific and public debate in recent years. Environmental organizations and some beekeepers suspect that these kinds of pesticide are the main cause of the bee problem and that the solution lies in banning them. Science has not yet provided an unequivocal verdict on the matter.

Yet for decades research has been going on at universities and government institutions in Europe and North America in the interests of the beekeeping industry. The main subjects of research are disease control, the food supply for bee populations and the impact of pesticides. But the beekeeping sector is not a rich one and this has an effect on the research, innovation and knowledge transfer in the field, explains Blacquière: 'There is plenty of room for improvement, including in the provision of infor- >

Dead worker bees at the entrance to a beehive, February 2013.

mation to beekeepers on topics such as disease control.'
What is more, scientists have not yet been able to determine with certainty the cause of death in a single bee or population. One of the reasons for this is that in the summer, bees only live for four to six weeks. So the bees that die off in the winter are not the ones that come into contact with the pesticides in the summer. Studies of the causes of bee deaths therefore focus on the links between possible causes and their effects on an entire population. Various different causes are being looked into, from pesticides to infectious diseases and the reduced supply of nectar and pollen.

DEFORMED WINGS

Blacquière: 'In international research, infections with the Varroa mite keep coming out as a possible cause of bee deaths.' The mite moved about 60 years ago from its original host, the Asian honeybee, to the western honeybee. It is just over one millimetre in size and feeds on the blood of larvae, pupae and adult bees. The Varroa mite not only sucks its host dry but also transmits a collection of insect viruses which affect the health of the entire population. These include DWV, which causes deformed wings and ABPV, which caused paralysis. Many of these viruses were already naturally present among honeybees but never caused problems. In an article in *Nature* (336:1304) in 2012, American and British researchers showed that the Varroa mite caused ever more aggressive virus strains to spread at lightning speed.
The Varroa mite is on the march all over the world, causing a systematic deterioration in the physical condition and lifespan of bees, as well as raising the risk of winter deaths. One source of this finding is large-scale monitoring of bee populations in several countries. A large Danish research institute, for example, monitored a total of 1200 beehives over a period of four years for the presence of pesticides and winter deaths. Statistical analysis of the data revealed a link between the seriousness of the Varroa infestation, the weakness of the population in the autumn and the number of winter deaths (*Apidologie* 41: 332). No link was found with the use of pesticides.

OXALIC ACID AND THYME OIL

Although a lot is known about the Varroa mite, it remains a key topic of international research, says Blacquière. The mite repeatedly develops resistance to pesticides, making it necessary to find new methods of keeping it under control. Previously successful chemicals such as fluvalinate and coumaphos either no longer work or are no longer permitted. Blacquière: 'Beekeepers now use organic acids and aromatic oils such as oxalic acid and thyme oil. But these have to be used with great precision, making their use often less effective than the simple chemical products of the old days.' Varroa mite control has become a standard aspect of beekeeping. There is still much to be gained by optimizing the way it is done, says Blacquière. Recent publications by the research group bijen@wur (*Journal of Insect Physiology* 59: 487), in which Blacquière is the senior researcher, show for instance that the assault on the bee's robustness made by a Varroa infection cannot be compensated for by putting enough food in the hive.
'The right timing of pest control seems to be crucial,' says Coby van Dooremalen, bee researcher at Plant Research International. She compared the effect of various different timings for Varroa control on the chances of surviving the winter (Public Library of Science ONE: *PLoS ONE* 7: e36285). 'Controlling the mite in July, before the winter bees hatch out, is more effective than doing it in September. Timely control of the Varroa mite extends the lifespan of the winter bees that are born in the autumn and raises the chances of the population surviving the winter.'

DIRTY HONEYCOMBS

The use of pesticides against mites (miticides) is reflected in the chemical analyses of beeswax, pollen and honey. This is because miticides are not biodegradable and accumulate up in fatty substances such as wax. Very high concentrations of miticide are sometimes encountered in beeswax, especially in beeswax from North America (*PloS ONE* 5: e9754). High concentrations of miticides or other pesticides in the wax can have negative effects on the bee population. These high concentrations can be formed when old honeycombs are melted down to create the basis for new honeycombs on which the bees can go on building. This is sometimes done for years in a row.
A number of other pesticides are introduced to the hive by the beekeeper in the interests of combatting bee diseases. The vast majority of the pesticides that arrive there, however, hitch-hike on pollens and nectar. Chemical analysis of wax and pollen from France, Germany, Spain and North America reveal a list of almost 100 different pesticides that the bee is exposed to. A bee population transports between 20 and 50 kilos of pollen to the hive, as a source of protein. The amount of nectar transported is in the region of 100 kilos.
The lifespan of bees that grow up as larvae in polluted honeycombs is four days – more >

The Varroa mite is on the march all over the world, raising the risk of winter deaths

BEE DEMOGRAPHICS

The development of a bee population is comparable with the demographics of a traditional farming community. Only after the passage of time do births, deaths, good harvests and bad weather result in a flourishing or declining community. Identifying the causes of bee deaths therefore means linking possible causes with their effects on the population as a whole.

The bee population through the year

Death ▶
At the end of the winter, with its cold and food shortages, about 10,000 of the original 30,000 bees are left.

◀ Reproduction
Reproduction
The queen bee lays her first eggs in January. By May she is producing about 2,000 per day.

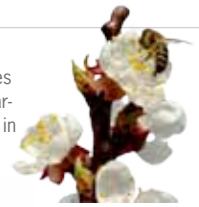
New bees
At the end of March the bee population is at its smallest. After this new larvae hatch faster than the bee death rate. ▶

Bee age
In the summer worker bees live for four to six weeks; in the winter six months. The queen continues to lay eggs in the summer to keep the bee population going.

Summer: 6 weeks

Winter: 6 months

Pollination ▶
Bees and bumble bees account for three quarters of the pollination in agriculture and horticulture.



◀ Pollen
A population transports tens of kilos of pollen per season, which mainly serves as a source of protein.

Honey ▼
A population transports nearly 100 kilos of nectar per year, turning it into honey for the winter.

◀ Swarming
In June, bee populations swarm in order to form new populations. The queen departs with half of her population.

Nuptial flight ▶
After the queen has left a new queen emerges. She sets off for a drone congregation area to mate.

◀ Drones and workers
Queens (1) and workers (2) come out of fertilized eggs. Unfertilized eggs produce drones (3).

Drone slaughter ▶
The task of the drones is to fertilize the queen. In August they are of no more use and are driven out of the hive and killed by the workers.

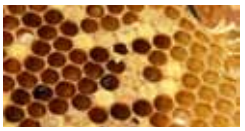
Hibernation
In October it is hibernation time. Bees do not sleep and keep the nest at 20 degrees.

Source: Bijen@wur

Threats to the bee population

Varroa
The Varroa mite feeds on bee and larvae blood, weakening the bee and also transmitting viruses such as DWW, which causes deformed wings, and ABPV, which leads to paralysis. Combatting Varroa is best done in July before the winter bees hatch, according to recent research. But the mite keeps developing resistance to pesticides.

Nosema
Parasites of the Nosema genus inhabit the digestive tract and damage the bee's stomach wall. Disinfecting the beekeeper's equipment and replacing the wax are effective preventive measures.



Foulbrood
Larvae can be affected by foulbrood – a collective term for various bacteria which can then affect the entire population.

Pesticides
While collecting pollen and nectar the bee comes into contact with almost 100 different pesticides which can cause damage either directly or indirectly. The substances accumulate in the honeycombs and other places. The life expectancy of larvae growing up in polluted honeycombs is 10 percent shorter, research has shown.

One-sided diet
Bees depend entirely on pollen for a number of essential proteins. The more varied their intake of pollens, the more likely the bees are to get all the nutrients they need. When the amount and variety of pollens fall short, the bees are more susceptible to diseases and winter deaths.

Scientists cannot yet rank the threats

Worker bees bring pollen and nectar back to the hive.

than 10 percent – shorter on average than that of normal bees, found an American study of larvae growing up in clean and in very polluted honeycombs (*PloS ONE* 6: e14720). Because different substances are in use in different countries, it is not easy to translate this study into European terms, says Blacquière: 'But it shows clearly that old, dirty honeycombs are not good for the bees' health. It is better to remove old honeycombs than to leave them in the hive for years.'

Fungicides are known to be relatively safe for bees. Their use is permitted during the flowering season and no field studies have ever been done on their effects on bees. Blacquière 'You can practically immerse bees in fungicides in lab tests without seeing any effect. But it seems as if there are nevertheless some effects on the food supply and the digestion of food.' Fungicides might inhibit the fermentation of pollen into ambrosia (or bee bread), the protein-rich food supply for the bee larvae. Lab studies by American researchers also show that fungicides can magnify the effect of other pesticides (*PloS ONE* 8: e54092).

NEUROTOXINS

In recent years beekeepers have increasingly suspected a link between neonicotinoids and the increased winter deaths. Neonicotinoids form a class of what are known as systemic insecticides. These insecticides can spread throughout the plant, which puts them at an advantage: a coating on a seed protects the plant from the time it germinates. But the neonicotinoids also end up in nectar, pollen, and drops of sap that sometimes hang off the ends of leaves. Neonicotinoids are neurotoxins that block neurotransmission in insects. They are deadly in small quantities for voracious leaf lice, and just as dangerous for the honeybee. This is why the use of neonicotinoids is subject to a whole range of regulations. For example: they may not be used on flowering fields or in an apple orchard in blossom.

In spite of all the regulations and restrictions, bees can end up being exposed to neonicotinoids both directly and indirectly during the course of a year. This came to light in 2008 in Germany, when thousands of bee populations lost vast numbers of bees in a short period in the spring. They had been exposed to dust particles containing neonicotinoids that came from coated maize seeds. Many of these populations recovered during the summer, however. Even when exposure does not noticeably lead to deaths, it can have negative effects: a substance that affects nerve stimuli could have more subtle effects on a bee's brain. Dozens of publications show effects of small quantities of neonicotinoids on learning behaviour, mobility and memory in bees. These are all characteristics that play a role in food gathering. In a recent study by French researchers published in Science (336; p 348), hundreds of honey bees were given drops of sugar water con-

taining a non-lethal dose of neonicotinoids. The skill with which they found their way back to the population was then monitored. The dosed bees performed poorly in comparison with bees that had not been dosed. Fewer of them found their way back and they tended to lose their way in new territory. Such losses can help to weaken a population.

CHRONIC EXPOSURE

Whether these effects correspond to what happens in winter bee deaths is unclear. Winter deaths are the endpoint of a development that takes place over several generations. In the summer a worker bee lives for five to six weeks, so thousands of bees die off in a population every week.
Whether extra losses of worker bees caused by contact with pesticides have a negative effect depends on many more factors. It is comparable with the demography of a traditional farming village. Births, deaths, diseases, good harvests and bad weather only reveal their impact over a longer period of time, in the form of a flourishing or declining community.
Blacquière's group is therefore doing a study, at the behest of the ministry of Economic Affairs, on winter deaths after chronic exposure to the neonicotinoid Imidacloprid. A large number of bee populations were given a sugar solution containing a non-lethal dose of Imidacloprid twice a week for four months of the summer of 2012. Blacquière: 'We are looking at the impact of this on the development of a bee population and ultimately on winter deaths. It is a large-scale and costly study involving 120 bee populations. We are still analysing the results.'
Because negative effects usually occur in combinations, this year we are going to study the survival of bee populations submitted to various combinations of Varroa infestation, intestinal infections with the single-cell parasite *Nosema ceranae* and exposure to low doses of neonicotinoids.

STREAM OF PUBLICATIONS

It remains difficult to pinpoint one sole cause of winter deaths. 'It would be nice if you could create a kind of ranking,' says Van Dooremalen. 'Then you could indicate the causes that should be addressed with high priority. But science hasn't got that far yet.' Internationally, bee research is generating a stream of data and publications from which it is not always easy to distil a clear and unambiguous picture. Research sometimes produces apparently contradictory results, regarding neonicotinoids for example. Sometimes tests done under laboratory conditions reveal clear negative effects while these are not clearly seen in terms of winter deaths in the hives. Epidemiological analysis offers a way of ordering all the data. This kind of analysis can be compared to proving that there is a relationship between smoking and lung cancer without doing experimental research. A fixed set of criteria known as Hills criteria are looked at, including the available knowledge on plausible disease mechanisms and the relation between exposure and disease or death.
In 2012 a study of this kind was published on exposure of bees to low doses of neonicotinoids through pollen and nectar and the increased number of bee deaths (*Pest Management Science* 68: 819). The researchers, James Cresswell, Nicolas Desneux and Dennis vanEngelsdorp from the UK, France and the US respectively, found no relation between neonicotinoids and the falling numbers of bee populations in various countries. Numbers had begun to fall before the use of these substances really took off in the mid-1990s. But the authors emphasize that the analysis is provisional. 'Our publication is primarily intended to illustrate the way you can evaluate the available knowledge in a systematic fashion,' says co-author vanEngelsdorp, a bee researcher at the University of Maryland. His view is that there is clearly something up and neonicotinoids could have a hand in it: 'I am open to all the possibilities. But personally I see a far clearer and more consistent effect of fungicides on bees in the research data. It surprises me, because why would that be?'
It remains a complex issue, says vanEngelsdorp. 'A lot of bee research is done on individual bees. It shows, for instance, that low concentrations of neonicotinoids are bad. What is lacking is evidence of effects at population level. That type of research is made more difficult by the robustness of the honeybee. A hive contains tens of thousands of bees and you can lose quite a number of individuals before the population is seriously affected. In that sense the honeybee is a tricky research model.' ■

Bee deaths dossier (A Dutch language page with links to some English articles): www.wageningenur.nl/nl/show/Bijensterfte.htm

EUROPEAN MORATORIUM ON NEONICOTINOIDS

The European Commission decided at the end of April to impose a moratorium on some of the ways three neonicotinoids – clothianidin, imidacloprid and thiametoxam – are commonly used. The restriction applies for two years and primarily targets uses which can cause honey bees to be exposed to the substances.
One of the applications concerned is the coating of seeds and the spraying of leaves of plants that attract bees, such as maize and oilseed rape. Other applications will still be allowed. In a report at the beginning of 2013, the European Food Safety Authority (EFSA) pointed out the risks that can result from the exposure of bees to neonicotinoids through pollen or nectar, but the authority believes there is not enough scientific knowledge to arrive at an accurate estimate of the possible effects on bees and other pollinators. Of the 27 EU member states, 15, including the Netherlands, voted for the proposal, 8 were against and 4 abstained. This distribution of votes does not provide a clear majority but in such cases the European Commission can impose a moratorium anyway. This applies from December 2013 throughout the European Union. During the moratorium the authorization criteria for these three substances will be re-examined.