Interaction between varroa and environmental factors

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The honeybee colony loss problem is multi-factorial, as often stated. Multiple factors may have some or even strong impact on honeybee colonies, and these negative impacts may contribute to colony losses. Some of the factors may come together (varroa and some viruses). As long as the relative contributions of the individual factors are not known it is wise to consider and take into account all possible causative factors. Meanwhile we should at least mitigate those factors that can be influenced or controlled.

It is however far too limited to put all factors that have ever been mentioned as a cause together in the basket with 'multi-factors', without weighing them. Which would enable any researcher to investigate his own factor of choice without discussion/dispute/controversies. But to make good progress researchers should do their best to find between the many multi-factors the "ulti-factor": the *ultimate* factor that is crucial for colony loss. This implies: through experiments eliminate the minor factors, and eventually only have left and focus on the few ultimate factors or the one ultimate factor. This is what bees@wur is executing through field experiments with (many) honeybee colonies using interaction experiments.

Of all factors which weaken honeybee colonies Varroa ranks immediately in the class of 'important causative factors', since varroa is always present and has proven to be damaging. In the "Honey-program", supported by the EU and the Dutch ministry of Economic Affairs, bees@wur works on varroa control. We are looking for factors that enhance the negative effect of varroa on bees and colonies, as well as factors that might compensate for the negative effect of varroa. Is a good provision with pollen able to mitigate the effect of varroa? Do other pathogens strengthen the effect of varroa? Are pesticides the ultimate varroa enhancers?

The multi factors

So Varroa is certainly one of the candidates that can contribute to colony losses. Another possible cause is shortage of food, quantity as well as diversity. Plant protection products, among which the neonicotinoid insecticides, may possibly play a role. Sometimes there is a suspicion of genetically modified crops, alike electromagnetic fields (for mobile phones and Wi-Fi). Also the veterinary medicines used by beekeepers (against varroa, and in some countries against American foulbrood and nosema) accumulate in the hive and may contribute. In addition there are many diseases and parasites that may harm colonies to varying extents. In the Dutch version of this article there is an (incomplete) listing, linking to Dutch references, including bacteria, viruses, protozoa, fungi and microsporidia, mites and insects.

Additive and synergetic effects

Besides the parasites varroa and nosema (*N. apis*) and maybe the bacteria that cause foulbrood diseases, as well as rare calamity events with plant protection products, the above mentioned factors seldom cause large honeybee colony mortalities. But of course there remains the possibility that the negative effects of individual factors added would together be too much for a colony, pushing the colony below its threshold of resilience and eventually to collapse. In such cases the factors are named additive. Sometimes however the effect of a combination of factors is greater than the sum of the individual effects. Then the factors are synergetic: they enforce each other's effects. The most obvious example is the synergism of varroa and the deformed wing virus (DWV): without varroa the virus hardly affects the bees, but in combination with varroa many serious symptoms occur, which weaken the colony. Moreover *vice versa* the effect of varroa on bees is much worse in the presence of DWV than in its absence. Chemicals can also strengthen each other's effects additively or synergistically: Hawthorne & Dively (2011) showed that the use of oxytetracycline (OTC, in America and other places used as antibiotic against American foulbrood) caused the bees to become ten times more sensitive to the anti varroa chemicals tau-fluvalinate (a.s. in Apistan) and coumaphos (a.s. in Perizin and Checkmite); obviously a synergism.

At last: the beekeeper

We should not forget our own role: by failures or mistakes we do often challenge our bees' resilience and put colonies at risk. In addition, factors like starvation, varroa infestation, chemical residues in the hive strike when we fail to feed timely, to control varroa adequately or do not exchange old combs with new foundation. Interactions between contributing causative factors are complicated as such, but the interaction with the beekeepers management reduces sight even more.

The parameters

Besides looking for factors that cause bee mortality it is important to look for parameters which give useful information. A parameter is in essence any measurable trait: for instance the weight of a bee, the colour pattern of a bee, the number of nosema spores in its gut. And on colony level: the number of (frames occupied with) bees, number of frames with brood, the level of varroa infestation. We are helped a lot by finding parameters that are easy to measure and deliver reliable objective data, but eventually the most important thing is that they do correlate with the mortality of colonies, and hopefully that it is logically explainable. Simply measuring and reporting ‘effects’ is not enough. We do know that good winter bees (essential for colony winter survival) have a higher level of vitellogenin than summer bees. It would therefore be plausible that when we distinguish colonies with high and low vitellogenin levels in autumn this is an indication for winter survival. But we can only be sure when we test for winter survival too. For that reason it is essential in addition to laboratory experiments, in which parameters can be easily measured, to carry out full field experiments with colonies. The latter should be continued over winter to be a real proof of effects on winter mortality of colonies, in order to correlate parameters and factors to these loss data.

Set up of the experiments of bees@wur

<u>One factor</u>

The past years we have executed (sometimes big) experiments aiming to measure the effects of a factor on a range of parameters in a bee colony, including survival over winter. The simplest experiments had one manipulated factor, while other factors as well as parameters were registered and followed. Such experiments provide a first indication which interactions occur between factors (even when only one was manipulated).

An example is the experiment (executed twice) in which the effect of the timing of varroa control during the summer was studied (Van Dooremalen et al. 2012). It was shown that early treatment against varroa (i.e. before the development of winter bees) was essential for a good winter survival.

In 2012 Sjef van der Steen executed an extensive experiment in which the only varied factor was the chronic feeding of the colonies with a non-lethal dosage of imidacloprid (one of the neonicotinoids) in sugar solution, compared to a control which was only provided the equal amount of sugar solution. In addition to winter survival also the colony development during the season, residues in the food stores in the hive, nosema and varroa infestation were monitored.

In 2011 Sjef van der Steen placed colonies on a rich forage site (Wageningen, Grebbedijk) and on a poor forage site (Planken Wambuis, Veluwe). On the poor forage area generally about half as many different species of pollen were collected compared to the rich site. The colony development and the colony vitellogenin level at the end of the summer was compared. These levels may relate to winter survival (see above).

Again in 2011 Tjeerd Blacquière, commissioned by the Wageningen University Science Shop and by ZonMw of the Dutch Ministry of Health and Sports, did an experiment to test possible effects of electromagnetic fields from an antenna on bees. The ubiquitous electromagnetic fields for Wi-Fi and mobile phone services might possibly affect the development and orientation abilities of honey bees. The parameters development of larvae to pupae to adult bees, the flight capacity of bees, the life expectancy of (winter-) bees, emergence weight and condition, failure of queens did not show differences. Only winter survival of the colonies seemed to be different, but this needs a larger experiment to be established.

An experiment by Bram Cornelissen in 2010-11 studied the effect of varroa control, in comparison with an untreated control, on the development of colonies, but also recorded infestations with DWV, ABPV, *Nosema apis* and *Nosema ceranae*. Development and survival of the colonies was negatively associated with varroa infestation, as well as with DWV infestation. Noteworthy the infestation with nosema was the highest in the largest and strongest colonies, which were treated against varroa. No relationship between nosema infestation and winter survival was shown.

Two factors

In the laboratory Coby van Dooremalen and others studied the effect of a combination of an infestation with varroa during the larval/ pupal stages combined with optimal versus sub-optimal pollen supply of the young just emerged bees. Abundant supply with pollen, be it very important for young bees, could nevertheless not compensate for the damage incurred by being parasitized by varroa (Van Dooremalen et al., 2013).

In an experiment in the field with the same factors in 2012 Coby van Dooremalen en Bram Cornelissen compared colony development and winter survival of colonies with and without varroa control, and with and without pollen trapping during half of the time. Varroa was de most discriminating factor for colony survival, but the limitation of pollen availability during the summer of 2012 almost prevented the colonies to build up a brood nest in the spring of 2013. There was just too little bee bread to start up, and the spring of 2013 was cold preventing the ability to catch up easily and quickly. Because all colonies without varroa treatment had died before the end of winter the interaction between varroa and pollen availability could not be fully explored.

An experiment by Sjef van der Steen tested the effect of pollen supply (with and without pollen traps in front of the hives) combined with chronic exposure to non-lethal levels of imidacloprid in sugar syrup. The idea was that possible negative impacts of a neonicotinoid would become more severe when foraging was hampered (in this case by reducing the amount of incoming pollen per trip).

Three factors

An experiment with three varied factors is running now from summer 2013 to spring 2014: (1: Varroa control yes and no; 2: Nosema inhibited with fumagillin yes and no; 3: chronic exposure to a sub-lethal level of imidacloprid, yes and no). In this experiment several aspects have been studied, including homing of (marked) foragers, flight capacity etc. Maybe the chronic exposure to imidacloprid may affect the resilience of the bee colonies against varroa or nosema. Next spring we will know more.

Many factors

The winter loss problem of honeybees is generally named multi-factorial. Nevertheless the most elaborate experiment is with 3 factors, not really multiple. However, an experiment with more than 3 manipulated factors on colony level is hardly or not manageable, let alone payable. And it has to be doubted whether it is possible to not get lost in the complexity. This implies that to elucidate the role of several possibly causal and interacting factors one has to take 'small' steps, and do several consecutive three-factor experiments.

Where do we stand now?

The discussion about the mortalities of honeybee colonies is fierce, and approached and performed from different positions. Attempts to rank the possible multi-factors that contribute to the mortalities all showed a dominant role for the varroa mite (Staveley et al., 2013, for mortalities linked to the Californian almond production; LeConte 2010, Genersch et al., 2010), a possibly important role for food supply (VanEngelsdorp & Meixner, 2010, Staveley et al., 2013), a less probable role for insecticides (especially neonicotinoids were studied; Cresswell et al 2012, Staveley et al. , 2013). A recent study from Belgium found correlations of mortality with varroa as well as with the infestation of *Nosema ceranae*. But in addition also the prevalence of *Crithidia mellificae*, a parasite that has up till now hardly received attention correlated with winter mortality (Ravoet et al., 2013).

Such studies and overviews give insight through correlations and weighing of correlations, but whether real causal relations underlie these correlations needs to be tested in experimental research, through multifactorial experiments on the level of colonies and apiaries. These are planned by bees@wur, and we foresee that we need quite a few experiments, since progress will be only achieved stepwise.

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