COLOSS – Varroa task force
WG 1: Comparison of methods to assess the Varroa mite infestation on honey bees

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Summary

Introduction

According to the COLOSS Workshop on "Varroa control strategies" of Bled, Slovenia, (May 22nd - 23rd 2014) we propose to compare and validate currently used methods to assess the Varroa mite infestation on adult honey bees and to recommend the method that is most reliable and simple to apply. The

Varroa mite infestation level can be assessed at an apiary level or at a colony level (e.g. in breeding programs with focus on varroa resistance or IPM strategies).

Several studies have been conducted on the subject of demonstrating that a soapy solution will effectively dislodge the mites after 30 minutes of shaking (Rinderer et al., 2004). Also the use of icing sugar has also been shown to be effective (Fakimzadeh 2000; Macedo et al. 2002; Lee et al., 2010a; Lee et al., 2010b) and in recent years has become the preferred method of beekeepers and technicians as it gives an immediate response and does not require collection of bee samples. All these methods evaluate the number of phoretic mites.

Natural mite mortality verified with the use of sticky sheets has been demonstrated effective but several conditions must be strictly respected to ensure reliability (Branco et al., 2006). Natural mite fall has been established by a formal Varroa working group (CA 3686, 1998) as a reference method in research to estimate Varroa infestation in a colony.

Aim of this WG is to compare different methods to assess the Varroa mite infestation on honey bees and also to collect the bibliography relative to studies in which the effectiveness of these methods (icing sugar, soapy solution) is assessed.

Requirements

In order to obtain reliable and comparable data we must meet the following requirements:

1. at least one apiary with at least 15 colonies (to be able to ensure final data from 10 colonies);
2. if possible 2-3 apiaries from same area (i.e. within 100 km from each other);
3. apiaries should be well described (geographic coordinates, colony disposition) and testers should be noted;
4. use only fully developed (productive) colonies (e.g. ten combs of bees and eight combs of brood in Dadant-Blatt hives);
5. use boxes with screened bottom floor to evaluate mite fall;
6. specify kind of box and type of bottom boards (i.e. Dadant-Blatt, 10 frames...);
7. use a scale to weigh samples in the field and specify kind;
8. bottom board wire screen must be clean of propolis and debris;
9. use the container indicated by the WG to collect the honeybee samples;
10. realize a video of the sampling method.

Materials

- Icing sugar
- 120ml container
- Jar (Dipl.-Ing. (FH) Harald Wössner, Julius-Leber-Strasse 12, 78652 Deisslingen, Tel.: +49 (0)7420 / 910 183, mail: harald.woessner@buero-hw.de)
- Sieves
- Dish washing soap
- Sticky sheets for bottom boards
- Kitchen / lab scale (accuracy min 1g)

Methods

1. Icing sugar sampling

A. Open the beehive;
B. Realize a measure of the strength of the colony with the Liebefeld method (link);
C. Select from the upperbox or the broodnest one external honey frame covered with an amount of foragers bees sufficient to fill a 120ml container; specify exact position of sample collection respect the distance from the brood frames (i.e. 1st = first frame near brood);
D. Weigh the bees, in the field before the icing sugar method estimation. If weight lower than 40g add bees.

From the frame realize one sample of bees

OPTIONAL (recommended for Institutes): collect multiple samples from each colony from different positions, to assess error due to uneven distribution of varroa in the colony. For each sample specify exact origin of collection.

Realize the icing sugar method estimation on each sample of bees (according to Macedo et al. 2002 and Danish method and Kirchain)

A. Add 35g of fresh icing sugar (approximately 2 tablespoons) into the jar;
B. Quickly pour the bees from the 120ml container into the jar;
C. Close the cap;
D. Gently rotate the jar with hands in 60 seconds in order to cover the bees with icing sugar;
E. Leave for 3 minutes the jar with bees in vertical position (cap up);
F. Shake violently the content of the jar (also with sidewall knock) through the screen lid, into a sieve that do not permit the passage of varroa mites. Shake for at least a couple of minutes.
G. Preserve the sample of bees freezing it in order to bring it to the laboratory and to check the accuracy of the method with a soapy solution wash (see point 2).

2. Checking the accuracy with a soapy solution wash

A. Prepare a soapy solution (max 5 ml of soap to one liter of water)
B. In laboratory[1] pour all bees to be tested into the beaker;
C. Add approximately 200 ml of soapy solution into the beaker;
D. Use a magnetic stirrer to shake the bees in the solution for 30 minutes at a speed of 900 rev/min;
E. Pour the content through two sieves (one to collect the bees and one to collect the Varroa mites); See type (link)
F. Subsequently perform a wash of bees left in the sieve with high pressure water to check the fall of other varroa mites remained between bees;
G. With the help of a lamp equipped with a magnifying glass, count the number of adult mites in the sieve;
H. Repeat the procedure until there are no more mites in the sieve;
I. Count the number of bees.

3. Natural mite fall evaluation

Evaluate the weekly natural mite fall starting 10 days before the icing sugar sampling with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions).
Take care to prevent access of varroa-eating arthropods. This can be achieved by:

- Cut grass
- If ants are present put the beehives on specific supports (i.e. legs of stands dipped in jars with water and an oily substance);
- Don't leave tray in place during season, insert only at the moment of starting to record mite fall. Sticky sheets work very well; specify whether use of sticky sheets or oily.

4. Phoretic and reproductive varroa mite amount evaluation

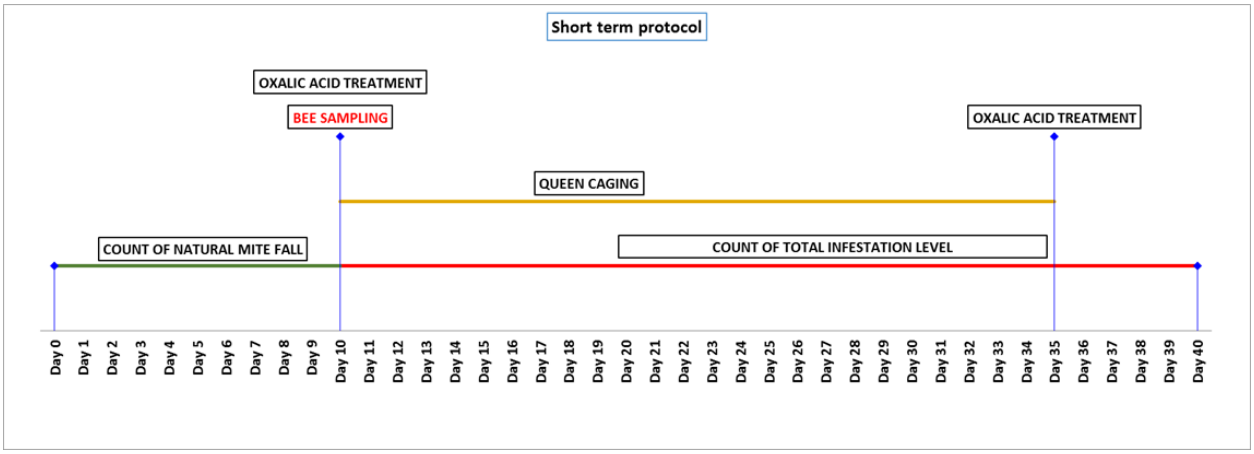
In order to obtain data about the total amount of varroa mites (phoretic and reproductive) inside the colonies and to correlate that data with the infestation level obtained with the samples, we propose two different protocols to be realized in the mid summer, after the main honey flow.

[1] If the laboratory equipments are not available you can use a whisk or an automatic beater to shake the bees in the solution for 2 minutes

The first easier to be applied and shorter, the second longest but with two samples of bees and that can permit a correlation between the different infestation levels and the acaricide efficacy of a formic acid treatment.

Short term protocol

From Day 0 to Day 10: Evaluate the natural mite fall (point 3) with sticky or oily label sheets on the bottom boards, replaced every 2 day (or adapted to local conditions)
Day 10: realize the bee sampling (point 1 and 2). Cage the queen (API.MO.BRU. Italian cage - link). Realize an oxalic acid treatment. Realize Liebefeld estimation.
[2] **See note
From Day 10 to Day 40: Evaluate the mite fall with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions) to evaluate the infestation level
Day 35: Release the queen. Realize an oxalic acid treatment[3]



[2] **Depending on beekeeper and researcher availability you can evaluate only the number of phoretic varroa mites:**
From Day 10 to Day 15: Evaluate the phoretic mite fall with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions) to evaluate the amount of phoretic varroa mites. In this case the protocol finish on day 15. (It will be called "VERY SHORT-TERM PROTOCOL")

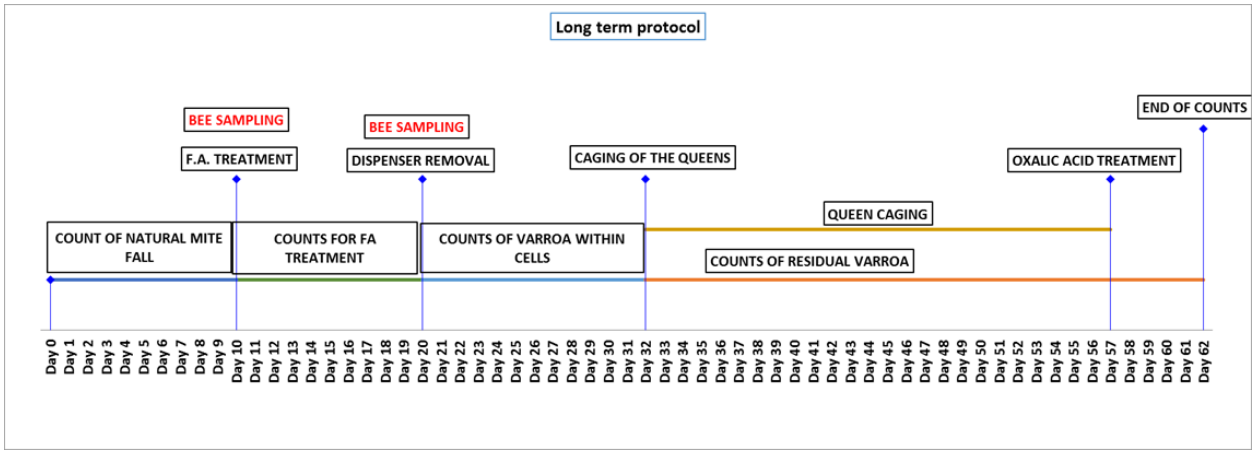
[3] Prepare the oxalic acid solution in this way: use 1 liter of hot water (70-80°C) to dissolve 100 grams of oxalic acid pure crystals. Then add 1kg of sugar (commercial one) to the hot solution. Then wait until it's cold.
With a syringe drop 5ml of the solution for each intercomb space occupied by bees.

Long term protocol

In this case we will integrate our protocol inside the that proposed in WG4 (Formic acid management) in order to evaluate the total amount of Varroa and also the possible correlation of infestation level with the acaricide efficacy of the treatment.

From Day 0 to Day 10: Evaluate the natural mite fall (point 3) with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions)
Day 10: realize the bee sampling (point 1 and 2). Realize the formic acid treatment (specification will be provided by WG4). Realize Liebefeld estimation.
From Day 10 to Day 20: Evaluate the mite fall with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions) to evaluate the varroa killed by formic acid.
Day 20: Remove the dispenser. Realize bee sampling (point 1 and 2). Realize Liebefeld estimation.
From Day 20 to Day 32: Evaluate the mite fall with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions) to evaluate the varroa died within cells.
Day 32: Cage the queen (API.MO.BRU. Italian cage - http://www.apimobru.com/). Realize Liebefeld estimation.
From Day 32 to Day 62: Evaluate the mite fall with sticky or oily label sheets on the bottom boards, replaced every 2 days (or adapted to local conditions) to evaluate the residual varroa.
Day 57: Release the queen. Realize an oxalic acid treatment

The protocol is described in the figure above:



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