

STANDARDIZED MANAGEMENT AND TEST PROTOCOL

- Beekeeper apiaries -

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Introduction

This study aims to compare colony performance, Varroa mite development and infestation rates under two different management strategies: conventional and innovative. The conventional method supposedly aims to control and minimize Varroa mite infestation at all times. In contrast, the innovative approach permits a certain level of mite infestation during the mating season, and thus, colonies that are able to produce healthy drones under infestation pressure, according to our hypothesis, are favoured by selection. To achieve this, winter treatments are omitted or minimized according to damage thresholds, and colonies are allowed to develop in spring without any Varroa control (also without drone brood removal). Effective summer treatments are applied, based on biotechnical methods of brood interruption, which minimizes mite infestation during the period of winter bee rearing and thus provides optimal conditions for overwintering. The ultimate aim of the field study is to demonstrate that colonies can be managed under the innovative concept without increased risk of winter losses, and without suffering disadvantages concerning management, health or performance.

Detailed research will be performed on an apiary network of Institutional apiaries across 11 countries. The main objective of this study is to understand the impact of an innovative colony management concept on overwintering, colony development, and Varroa population growth. Several apiaries within this study will also diverse additional roles, such as providing samples for studies on the holobiont, immunity, pathogens, and pollinators. Approximately half of the colonies involved in this network will be equipped with digital scales, and some will also have bee counters, contributing to the establishment of the Pan-European Digital Apiary Network.

Your involvement as a beekeeper citizen scientist in this study is highly appreciated. You will have a unique chance to test an innovative colony management approach showcasing its benefits to the broader beekeeping community. Further, your records and feedback will give valuable insight into how the proposed method works within the realm of practical apiary conditions. Finally, we are sure that your experience gained during this study will provide additional recommendations for us, which could be used to improve and adapt this method to local conditions of your region.

Together, we're not just exploring new frontiers in beekeeping; we are setting the stage for a sustainable future for honey bees.

COLONY SETUP

Starting Conditions

- There are no strict rules on starting conditions of colonies used in study. However, there are two recommendations:
 - Start experiment with colonies of same origin (e.g., new colonies either formed from brood combs or shook swarms, colonies from previous season etc.).
 - Try to use colonies of similar strength in terms of bees and brood population so that we avoid difficulties in making conclusions that may arise if some colonies are much weaker/stronger at the beginning of the study
- There is no specific number of colonies needed to start the experiment, you can even test the method on a single hive! But if you want to contribute statistically meaningful data, the suggestion is to start with a total number of 10 to 20 colonies.
- Number your colonies in a durable way. This helps keeping track of the treatments, infestation levels and fate of the colony.

Experimental Groups

- Forming two experimental groups is recommended, however not mandatory.
- Distribute colonies into two experimental groups of equal size (for example 5+5).
- If possible, keep experimental colonies at the same apiary (under the same environmental conditions and in the same type of hives). If this is not possible, try to equalise distribution of groups across apiaries.

COLONY MANAGEMENT

Conventional group

- Manage colonies in your usual way
- Apply your commonly used treatment regime against Varroa, and record it in the Recordkeeping card.

Innovative group

- No Varroa control (also no drone brood removal) during the spring development
- Biotechnical summer treatment – the same method must be applied to all colonies of this group. Various methods are available – detailed information and descriptions can be found in the article “Summer brood interruption as integrated management strategy for effective Varroa control in Europe” (Büchler et al., 2020; Link: t.ly/jzMYU); however we suggest a brood break by queen caging for 25 days followed by oxalic acid treatment with authorised products, according to national legislation. You can contact your local BeeGuards partner (www.beeguards.eu) to discuss the different methods and the best suggested timing to apply them under your local conditions.
- Threshold based treatment (winter treatment)
 - Try to avoid winter treatment unless the infestation rate of adult bees reaches a threshold of 3-4 % in late summer (August – September for central European countries)
 - Colonies that reach these threshold values are recommended to be treated following the regular winter treatment approach (like oxalic acid trickling or other certified treatments, during broodless condition in November/December).
 - If a colony is treated, it remains in the same group, and measurements follow on because threshold-based treatment is part of the concept. It is important to record the date of treatment and what kind of treatment is used.

Colonies should be managed in a way to prevent swarming, as this will cause a brood break period early in the season and reduction of colony strength and Varroa infestation. If you regularly make splits by removing brood combs or worker bees, you can follow this approach, however, do this on all colonies. So, you can eventually remove, but please do not add any brood/bee frames to the colonies.

DATA COLLECTION

In Figure 1 the complete and optimal participation is depicted, however, modular participation is also envisaged, so any beekeeper can participate as long as they provide one of the measurements here described.

The experiment is envisaged over two seasons (but a single season is also possible). Testing could start in 2024 or 2025. In case of two subsequent testing seasons, the initial colonies or new (different) colonies could be used for the second season.

The complete set of measurements includes:

- Evaluation of colony strength
- Honey yield
- Swarming behaviour
- Infestation of colonies with varroa mites

Each kind of measurement is described in detail after the Figure 1 and will also be illustrated with different kinds of media (check out the [BeeGuards.eu](https://www.beeguards.eu) website for updates!).

Implementation timeline

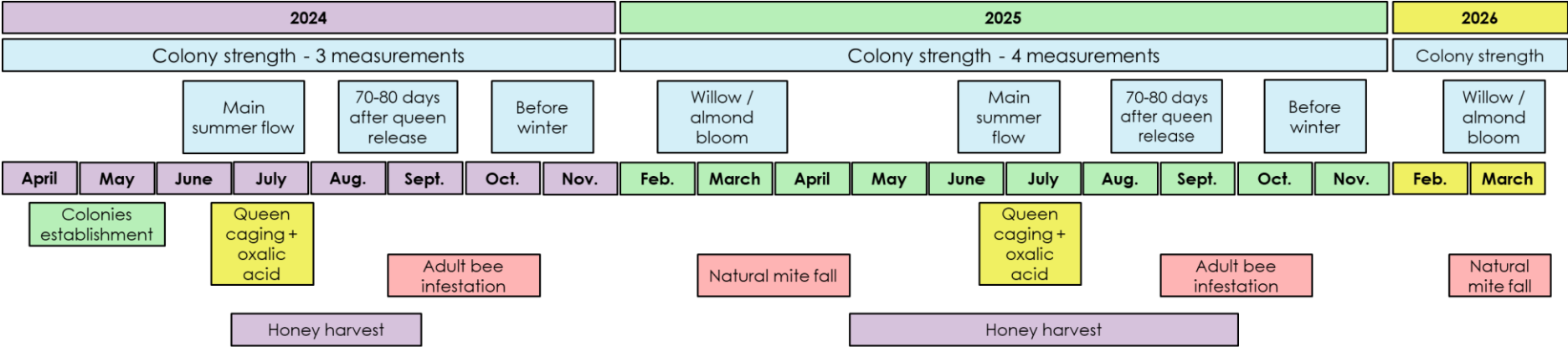


Figure 1. Timeline for implementation of all planned activities – WP1 – BeeGuards field study – Beekeeper apiaries.

EVALUATION OF COLONY STRENGTH

As shown in Figure 1., strength of the colonies is evaluated three times in Year 1, four times in year 2, and once in Year 3. Adjust this to regular colony inspections that you commonly perform at your apiary:

1. First spring inspection (willow/almond bloom).
2. During main summer nectar flow.
3. Autumn inspection (approximately 80 days after the queen was caged in the innovative treatment. At this moment, we don't expect any more negative effect of brood break on colony strength).
4. Pre-winter inspection (your usual last inspection of colonies before wintering starts).

Evaluation of colony strength is done by simply counting number occupied bee lines.

- Open the colony, and with limited use of smoke estimate number of bee lines (space between frames) occupied with bees in a box (Picture 1).
- Repeat this for every box and super.
- Record it in the Recordkeeping card.



Picture 1. the red rectangle shows one "bee line" (space between frames occupied with bees)

HONEY YIELD

During each honey harvest, estimate the production of honey by assigning grades: +, 0 or – (+ better than colonies of the other group, 0 same, - worse). The honey yield can also be given in measured or estimated kg. Record it in the Recordkeeping card.

Besides honey, please record any other production as well (i.e. pollen, splits/nucs, etc.), separately for each experimental group.

If there are no experimental groups, this evaluation is not performed.

SWARMING BEHAVIOUR

Manage the colonies in your usual way to prevent swarming. If the colony swarms, it should be noted in the Recordkeeping card.

Note any eventually removed frame.

INFESTATION OF COLONIES WITH VARROA MITES

Natural Varroa mite mortality - spring (during willow or almond bloom)

To measure the natural mite mortality, colonies need to be equipped with a screened bottom board (Picture 2). The screen should cover the full area of the bottom board. Natural mite mortality should be checked during a 3-week period in the spring (3x7 days). This is important to gain insight on the mite fall after the wintering period.

Our suggestion is to monitor the mite fall even more frequently to avoid any problems that may result from high Varroa mite infestation of the colonies.

The procedure is as follows:

- use a sticky sheet or a bottom board covered with oil (biodegradable chain saw oil works well)
- insert the sticky sheet or bottom board under the colony
- after 7 days remove the sheet/board and count the number of fallen Varroa mites, put new sticky sheet or bottom board
- calculate the number of fallen mites per day
- record it in the Table 1.



Picture 2. Tool set for fast preparation and cleaning of inserts in the screen bottom board; spray bottle for oil and a spatula to quickly clean the insert

Adult bee infestation – Summer/Autumn

To determine the infestation rate of bees, a sample of bees is collected from the honey chamber of the hive in a 100 ml plastic cup. During the early and late season, when the honey chamber is not separated or populated, take samples from the side combs away from the brood nest and be careful not to sample the queen.

To evaluate the infestation rate of colonies based on this sample, we recommend to use the Powdered (icing) sugar method, or the soapy water wash or alcohol wash. If applied correctly, both methods gain comparable results and thus you may choose the one that fits best to your working style. However, be aware that the icing sugar method will only work well when all components (the sugar itself, but also the bees and frames) are completely dry. Do not use this method in humid conditions or during a strong nectar flow.

Both methods of sample processing are described below:

Powdered (icing) sugar method:

For this evaluation, you will need:

- Plastic sheet size of 40x40 cm.
- Small jar of size 100-120 ml for sampling (e.g., urine cup)
- Big jar for shaking (minimum size of 750 ml) with fixed metal mesh (size 2.8 mm) on the jar lid or on the bottom of plastic jar
- Icing sugar. On average you will need 250 g for 7 colonies.
- Tablespoon.
- Very fine sieve (through which Varroa cannot go through)
- Table 3.1. – to insert data in the field

- Colony's recordkeeping card

Procedure:

- Take a frame from the honey chamber and shake the bees on the plastic sheet. If a queen excluder is not used, take the outer frame from the top box and be careful not to sample the queen.
- Fold the sheet and put bees into the small jar until it is full. When a 100 ml jar is full, it contains around 50 g of bees, which is approximately 450 bees. For this reason, it is important that the jar is always full of bees when taking samples. Otherwise, you should weigh each sample of bees you take.
- Transfer the bees into the big jar with a meshed lid or bottom (Picture 3)
- Insert 5 tablespoons of powdered sugar and gently shake the jar so that all bees are covered (Picture 4)
- Leave it for 3 minutes in the shade with occasional shaking
- Invert the jar with bees and powdered sugar and shake it over a fine honey sieve for one minute (Picture 5)
- Count the mites (Picture 6)
- Record the score in Table 3.1.
- Return the bees to the colony
- Calculate the infestation rate by following formula:

$$\text{Bee infestation} = \frac{\text{number of mites}}{450} \times 10$$



Picture 3. Sample of bees placed in a jar for shaking.



Picture 4. Bees covered with powdered sugar.



Picture 5. Shaking the mites out of the jar.



Picture 6. Mites are found on the sieve

Soapy water or alcohol wash method

For this evaluation, you will need:

- Plastic sheet size of 40x40 cm.
- Small jar of size 100-200 ml for sampling (e.g., urine cup)
- Portable kitchen scale
- Big jar of approximately 400 ml for shaking (e.g., 500 g honey jar)
- Dish soap or ethanol (pure or denaturated, e.g. methylated spirit)
- Fine honey sieve (through which Varroa cannot go through)
- Table 3.1. – to insert data
- Colony's recordkeeping card

Procedure:

- Note colony ID and date on the small jar
- Take a frame from the honey chamber and shake the bees on the plastic sheet. If a queen excluder is not used, take the outer frame from the top box and be careful not to sample the queen.
- Fold the sheet and put bees into the small jar until it is full.
- Close the lid and freeze the bees.
- After collecting the bees as described above, store the samples in the freezer (-18 °C) until analysis.
- To dislodge mites from bees, use the soapy water wash method as follows (soapy water may be replaced by ethanol - denaturated or pure).
- Put the big jar on the scale (marked with colony ID and date as given on the small jar)
- Tare weight to "0" (Picture 7)
- Transfer sampled bees of the respective colony from the small jar into the big jar
- Record weight of bees and date of sampling in Table 3.1.

- Add some drops of dish soap and fill the glass with water
- You may prepare several samples like this before continuing with the next steps (mind to note colony ID and date on each jar!)
- Stir bees every 5 minutes for 30 minutes to dislodge mites from bees (picture 8) or use a mechanical laboratory shaker
- Transfer the sample to the upper (bigger) part of a double honey sieve and wash it using the shower (picture 9)
- Record the number of mites found on the lower (smaller) sieve (picture 10) in Table 3.1.
- The infestation rate is determined by the following formula:

$$\text{Bee infestation [\%]} = \frac{\text{number of mites}}{\text{weight of the sample}} \times 10$$

Use “Table 3.2. Varroa mite wash (Calculation)” on your computer to easily calculate the bee infestation

The infestation rate is presented as the number of mites per 10 g of bees, which we use as an approximation of the percentage of mites.



Picture 7. Weighing of bee samples



Picture 8. Washing bees in water with few droplets of dish soap. Stirring is done every few minutes for 30 minutes.



Picture 9. Washing bees through double sieve.



Picture 10. Separated mites on the lower sieve.