

SEASONAL BROOD INTERRUPTION STUDY 2016/2017

Coloss Varroa control task force, WG2
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INTRODUCTION

In many regions, long reproductive seasons cause strong Varroa population increases which often result in colony losses. An artificial interruption of the seasonal brood cycle can be a useful option to reduce varroa infestation levels in due time. During broodless periods all mites are forced in a phoretic stage and can easily be trapped with open brood combs or killed by varroacides (f.e. oxalic acid), which were primarily developed for winter treatment.

During the spring workshop in May 2016 on Unije, WG2 members decided to organize an international study during season 2016/2017 to estimate the effects on **colony development** and **Varroa treatment efficacy** of different brood interruption techniques and oxalic acid application methods under different environmental conditions. Basic descriptions of the working procedures for the following methods:

- I. **Queen caging and treatment,**
- II. **Total brood removal combined with trapping comb and**
- III. **Classical trapping comb technique**

are appended to this protocol.

In order to achieve comparable data for a common evaluation and scientific publication each participating partner needs to test at least 2 groups of 10 colonies each according to the following protocol. All data have to be reported until April 2017 and will be revised during a workshop in spring 2017.

EXPERIMENTAL DESIGN

Material

- An apiary of at least 20 full size, naturally infested colonies.
- Varroa control cages and excluder frames (see basic method descriptions).
- Sticky sheets (sprayed with vegetable oil) for counting varroa mites on bottom board.
- Apistan, Amitraz, Bayvarol or CheckMite strips for critical control treatment.

Colony conditions

- Create two or several groups of at least ten colonies each. Data on colony strength (Liebefeld check) and mite infestation level of bees, as measured at the beginning

of the experiment should be used to distribute the colonies to homogeneous groups.

- Take field measures to minimize drift and reinvasion.
- Remove or pre-treat colonies not included in the trial.
- Limit colony handling as far as possible. Do not affect natural bee population growth and brood development.

Treatment variants

1. Caging, OA trickling 4.2%

Queens are caged for 25 days, 4.2 % oxalic acid solution (prepared with 10 g oxalic acid dihydrate + 100 g sucrose + 100 ml water) is administered thereafter by trickling, 5 ml/comb. Alternatively, Apibioxal® solution, which contains 4.2 % oxalic acid can be used by trickling 5 ml/comb according to label instructions.

This variant will be used as a standard on all involved apiaries. Choose one or more of the following treatment variants for comparison to this standard:

2. Caging, OA trickling Oxuvar®

Queens are caged for 25 days, Oxuvar solution according to label is administered thereafter by trickling, 5 ml/comb.

3. Caging, OA sublimation

Queens are caged for 25 days, 2 gr Oxalic acid dihydrate crystals are administered thereafter by sublimation with the VARROX®-vaporizer according to label instructions.

4. Total brood removal

All brood combs are removed according to the described working procedure.

5. Classical trapping comb technique

The queen is caged on single combs which are removed after sealing, over a 27-28 day period, according to the described working procedure. Note the use of either drone or worker brood as trapping combs and the rhythm of comb change (4*7 versus 3*9 days) in the protocol.

6. Trapping comb technique “simplified” (1*20 days)

The queen is caged on a single comb foundation, which is removed after a 20-days period. Transfer the queen in the nest. Take the comb foundation on which the queen was caged, count varroa mites in the sealed cells and destroy the comb. After 5 more days, realize the oxalic-acid treatment on the colony.

7. Local standard treatment for colonies with brood

A local standard treatment for colonies with brood, like the use of formic acid or thymol, may be included as an extra group in the experiment.

Critical treatment

All colonies have to be treated with either Apistan, Apivar, Apitraz, Bayvarol or CheckMite strips according to label instructions. The product is chosen with regard to the legal local registration and the presence of resistant mites.

In the case of any pharmacoresistance a combined use of two of these drugs is recommended to ensure a high efficacy level.

Timing

Start all groups at the same date and synchronize also the critical treatment.

The starting date depends very much on the local conditions. The honey harvest has to be finished before the treatment with OA on day 25. The handling of the colonies in the beginning of the experiment (caging of queens, total brood removal) is easier if there is some natural nectar flow available.

DATA COLLECTION**Colony performance**

At the start of the experiment, check all the colonies and measure adult bee population and brood area with the Liebefeld method. Repeat the measures about 4 weeks after the start of the critical treatment, pre- and post-wintering.

In all colonies, take note of queen mortality/supersedure during the experiment.

If a honey flow is likely to occur during the treatment, weight the colonies at the start of the experiment and weight again post-harvest.

Mite infestation

To realize homogeneous groups for the varroa infestation level, take a sample of about 500 bees (50-60 gr) from each hive to determine the bee infestation level at the beginning of the experiment by the washing or powder sugar method. Alternatively, count varroa natural falls for 2 weeks before starting the experiment.

Insert sticky sheets on all hives at the beginning of the experiment. Count the mites in intervals of 3-4 days, if possible. However, some flexibility is given about the intervals to adapt to the local working conditions.

The sticky boards are used until 2 weeks after removing the strips for the critical treatment.

As an optional information all removed trapping combs can be checked for mite infestation by uncapping and washing out the brood. The mites can be separated from the brood by the use of honey double sieves.

Requeening

Especially for large enterprises it might be attractive to combine the caging technique with requeening. If you can do so within this experiment collect data on the practicability and efficacy (queen acceptance rate) for one of the following procedures:

- Kill the old queen instead of caging it and wait for supersedure by an emergency queen. Treat with OA 25 days after removing the old queen.
- Kill the old queen immediately or after some days of caging, 7-9 days later destroy any supersedure cells and introduce a mature queen cell. Treat with OA on day 25.
- Cage the old queen, remove it after 25 days and introduce a young mated queen in a shipment cage under candy closure to be released by the bees within the next 12 hours. At the same time treat with OA.

Take note of the queen age

As an optional information, take note of the queen age. The success of the last method probably depends very much on the physiological status of the young queen. If possible take young queens directly from their mating boxes and introduce them to the colonies within the same day.

Additional recordings

Locations

Measure the GPS coordinates and the altitude of the apiary. Environmental recordings f.e. on nectar and pollen flow are useful to frame the trial correctly and to compare results between apiaries.

Climatic conditions

If possible, take continuous recording of external and internal temperature and relative humidity (f.e.: iButton Hygrochron Temperature/Humidity Logger DS1923).

Bee mortality

Dead bee counts may be taken with traps placed at the hive entrance. Note any obvious mortality of brood or adult bees occurring during the experiment.

Bee diseases

Note any symptoms of brood or bee diseases occurring during the experiment. If it is possible to arrange a labor analysis, bee samples for virus and Nosema control should be taken in autumn.

Residues

If it is possible to arrange a residue analysis for oxalic acid, honey, bees and wooden frames may be sampled a few days after OA treatment and again about 8 weeks after OA treatment.

Work load

Make notes on the time effort for the application of the different treatment variants.

Online database

Alex Uzunov will develop and share an online database to collect all relevant data for a common evaluation of the experiment.



Calendar of main activities

DAY	Caging methods	Brood removal	Classical Trapping comb	Trapping comb "simplified"	Sticky sheets *)
0	Liebefeld check Bee sample for Varroa infestation check				Insert sticky sheets
	Queen caging	Total brood removal	Queen on TC-1	Queen on TC	
3					
6					Change
9		TC removed	Queen on TC- 2		Change
12					Change
15					Change
18			Queen on TC- 3, TC-1 removed		Change
20				Queen release, TC removed	
21					Change
25	Queen release, OA administration			OA administration	Change
27			Queen release, TC-2 removed		Change
29					Change
32					Change
35			TC-3 removed		Change
	Insert strips (critical treatment)				Change
38					Change
42					Change
45					Change
49					Change
52					Change
56					Change
59					Change
63					Change
66					Change
70	Liebefeld check				Change
73					Change
77	Remove strips (critical treatment)				Change
80					Change
84					Change
87					Change
90					Remove sheets
pre- winter	Liebefeld check				
post- winter	Liebefeld check				

*) : If possible, sticky sheets should be exchanged in 3-4 day intervals but there is some flexibility to adapt the timing to local conditions

APPENDIX: WORKING PROCEDURES

I: Queen caging and treatment

Use Var-Control cages , 5 x 7.5 cm, with queen excluder on both sides¹. Fix the cage in a comb. This frame can be left in the colony for re-use.

Refer to the following pictures. Locate (1) and gently remove (2) the queen from the comb. Introduce in the cage (3), shut firmly (4) and leave untouched for 25 days. On day 25, unseal the cage (5) and reintroduce the comb into its slot (the queen finds her way out shortly). Administer the oxalic acid solution onto the combs (6).



1) Locate the queen.



2) Remove the queen.



3) Introduce in the cage.



4) Shut the cage.



5) On day 25: open the cap.



6) On day 25: oxalic acid administration.

¹ The normal queen excluders used for supers (slots of 4.2 mm) occasionally let tiny queens pass through. These slots of 3.7 mm are safer: <http://www.apimobru.com/en/index.htm> (a device for beekeepers not familiar with queen handling is shown as well).

II: Total brood removal combined with trapping comb

(see also: <http://teca.fao.org/read/8401>)

Step 1

Open the colony, find the frames with brood (open and sealed), shake the bees from them (Fig. 1 & 2) and dispose them in a new hive body or take them home for melting. Make sure that the queen remains in the original hive.

Note I: for establishing a new colony from those frames -, leave about 500 worker bees per comb for warming the brood. The brood combs from different colonies can be put together in a new hive on 1 – 3 supers (10-30 frames). Make sure that sufficient food combs are added to supply the bees for the next few weeks. The brood collector should be moved to a separate apiary to avoid robbery and mite exchange with the original hive. If no queen is added, the bees will rear an emergency queen. After 21-24 days all the brood is gone and the bees can then easily be treated against *Varroa*.

Note II: all brood from the colony should be removed.

Step 2

Insert frames with build combs or foundations (Fig. 3) on the free places of removed frames. Leave free space for a single frame.

Step 3

Insert a single trapping frame (Fig. 4) with all stages of open brood. If there are any sealed brood cells present which could emerge within the next 9 days they have to be destroyed.

Note I: in case of intensive honey flow the honey suppers should remain on the hive.

Step 4

After 7-9 days, open the colony and remove and count varroa mites in the sealed brood. After that, destroy the single trapping frame now with sealed brood and confined mites, add an empty comb or foundation to replace the trapping comb.

Step 5

Without regular nectar flow (rainy weather f.e.) there is need for colony feeding with 2 - 3 liters/colony/week.



Fig. 1 Removal of frame with sealed brood



Fig. 2 Establishment of new colony



Fig. 3 Insertion of frames with foundation

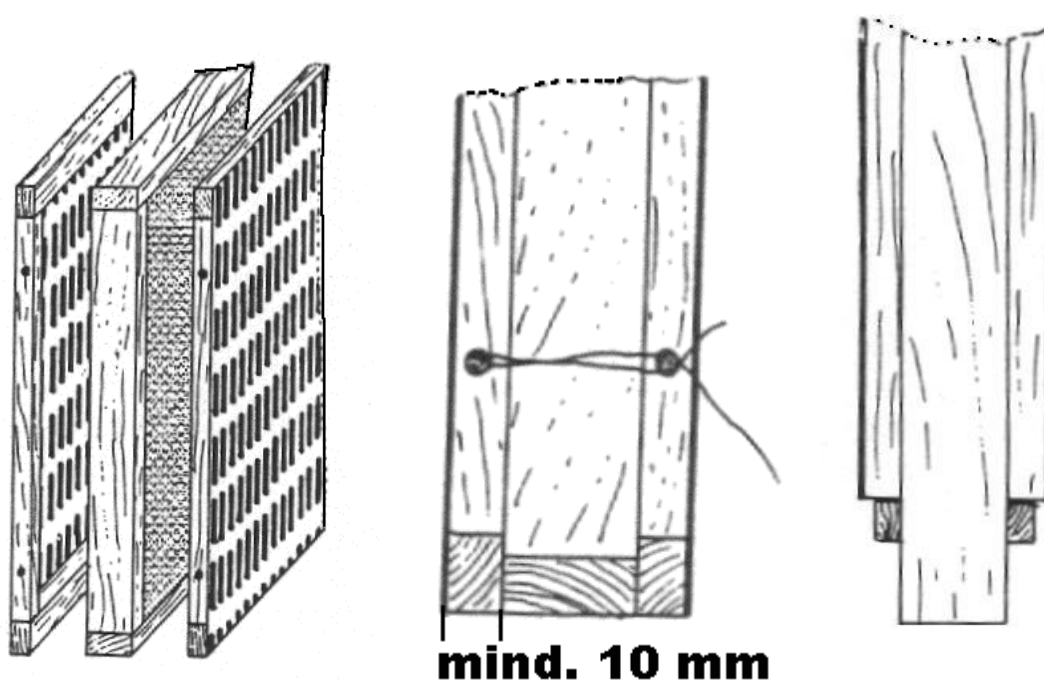


Fig. 4 Trapping frame with open brood (centre position) and frames with foundation and food

III: Classical trapping comb technique (3*9 days)

The classical trapping comb technique was already developed in the 1980ies by Maul et al (see: Papers trapping comb technique.pdf) and proofed to be a highly efficient biotechnical control measure which allows to fight Varroa without the use of any chemicals.

The queen has to be caged for subsequent intervals of 3 * 9 days or 4 * 7 days on one empty drone or worker comb per interval. For this purpose simple excluder frames can be constructed by the use of 10 mm wooden laths and plastic or metal queen excluders which fit exactly to the combs frames (1).



- 1): Construction of excluder frames to be fixed on both sides of a standard drone or worker comb

Before you start to search for the queen a trapping comb is prepared with a central hole and an excluder fixed on the backside (2).

As soon as the queen is found put it carefully through the hole to the protected backside of the comb and place the second excluder frame on the front side of the comb. A thin wire or a pair of rubber bands can be used to tightly fix the excluder frames on both sides of the trapping comb. Check carefully for the fit of the excluders to be sure that there are no larger gaps at the edges of the combs through which the queen might escape.

The trapping comb is returned to the central brood nest. After 9 (or 7) days the comb is taken out, opened and the queen is transferred to a second trapping comb in the same way as described above. The first trapping comb which should contain all stages of open brood is marked and put back to the brood nest. Another 9 (or 7) days later the first trapping comb is completely sealed. It will be removed and melted to kill the mites trapped in (3).



- 2) Empty comb with a central hole and queen excluder frames prepared to introduce the queen



- 3) After 7 – 9 days the trapping comb should contain all stages of open brood (left). After moving the queen to the next trapping comb, it remains in the brood nest until all brood is sealed (right).

After 27 (3*9) resp. 28 (4*7) days the queen is released. At that time old comb's can easily be replaced by new combs or foundation-combs. The last trapping comb is removed after another 9 (or 7) days.

Depending on the season, colonies may build some supersedure cells on the remaining open worker brood during the first trapping period of the queen. To avoid any risk for the old queen the colonies may be checked and any supersedure cells be destroyed during the next inspection (7-9 days after the start of the treatment).



IV. Trapping comb technique “simplified” (1*20 days)

The queen is caged on a single comb foundation for 20 days (use excluder frames as described before). After 20 days the comb is taken out, opened and the queen is released to the nest.

Take the comb foundation on which the queen was caged, count varroa mites in the sealed cells and destroy the comb. After 5 more days, realize the oxalic-acid treatment on the colony.