

PROTOCOLS TO COMPARE FORMIC ACID TREATMENTS
Working Group 4 of COLOSS Varroa Control TASK FORCE

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Active compound to study: formic acid, in association with oxalic acid as a control treatment.

Apiaries:

Locations: The apiaries should be located at different altitudes (coastal plain, hill, mountain) or at least different climatic conditions (sunny place or close to a forest etc...).

The aim is to cover a wide range of conditions in different countries to be able to compare the efficacy of the Varroa treatment.

Each apiary: Minimum 5 hives/treatment type. Minimum 10 colonies per apiaries. The more the better. We maintain the number rather high because we want to use the Liebefeld methods (<http://www.agroscope.admin.ch/imkerei/00000/00294/02107/index.html?lang=en>) to verify the toxicity on the honey bees to estimate colony strength and if treatment affect colony development and strength.

2 “mandatory” treatments: formic acid using the dispenser Nassenheider Evaporator Professional; MAQS

Other dispensers (1 or 2 or more) of your choice, including locally used dispenser Type if apiary has enough colonies: Generally there are 2 principles with dispensers. The one with a sponge system, the one where the acid is contained in a bottle. For example in CH will be studied: FAM (local type, sponge system) and Liebig Dispenser (Bottle system).

Please consider the different dispenser principle when selecting the dispenser of your choice. Ideally you would test the two different principles.

Hives: Specify the type of hives that will be studied in your respective country.

For example, in Switzerland there are 2 types of hives mainly used: Swiss hive in beehouses and Dadant. Studies will be performed on this two different types hives. In different countries different hive types may be tested.

Experimental groups: (5 hives/dispenser)

The treatments and the dispensers will always be realized according to the manufacturer user manual.

For the test, no supers! Acid formic is supposed to be used after harvesting anyway.

Consumption of Liquid formic acid should be measured: if there is rest in the bottle after the treatment, measure the rest.

In MAQS manual, According to manufacturer, you can use supers. This can have consequences because of the increasing of formic acid levels in your honey. Therefore we will test MAQS without supers. It is also said you can remove the strips or leave it after 7days. In this trial we will REMOVE the strips after 7 DAYS of treatment!

If you really want to realize test with supers, you should include in the study the consequences for the honey produced after the treatments, like: taste of honey, increasing in free acids equivalents and formic acid, hydroxymethylfurfural (HMF) values.

Remark: It is recommended to feed the colonies before formic acid treatment.

Treatments:

1) Nassenheider Evaporator Professional (about 10 € each evaporator; provided with a bottle with visible filling level). Formic acid 60%. Dosage: 180 ml/treatment for 10 days treatment.

2) MAQS. 7 days of treatment, according to label indications.

3) Optional: Your own local dispenser (e.g. FAM dispenser: Formic Acid 60%; Liebig: Formic acid 85%; BLV in Italy).

Monitoring of T° and H°:

T° and H° will be monitored by one logger/apiary placed at 2 meters, in the shade, protected from the rain.

Proposed logger model: Ibutton Model: DS 1923 (cost: Maximum about 70 € a piece)

You will need to buy as well a cable to read them: DS1402D-DR8 and an 1-Wire USB Adapter (cost: about 15 €).

Or something with the same technical requirements /resolution (e.g. EL-USB-2+)

We also decided to have one logger per colony.

After the experiment each logger should be recalibrated to check if they are still working properly. If there is deviation a correction coefficient should be applied to the collected data.

Humidity and Temperature will be recorded every hour during the treatment time.

The logger should be located in a way that it is protected from the bees, not too close from the dispenser, but still close enough to register realistic temperature.

Here some picture to show where to put the logger.

Figure 1: Protection of the logger (left). Plastic cap + net + caoutchouc ring + Ibutton (right)

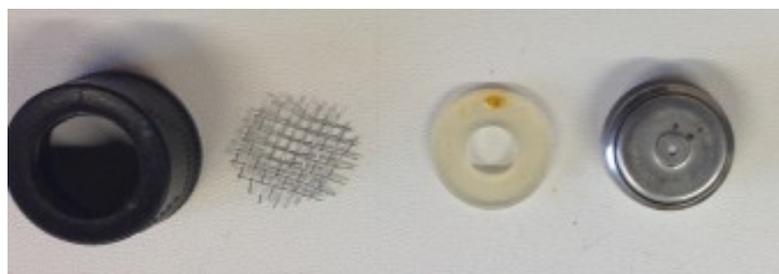


Figure 2: Location of the logger on the FAM



Figure 3: Location of the logger on the Nassenheider Pro



Figure 4: Location of the logger on Liebig



Figure 5: Location of the logger for MAQS



You can adopt 2 different protocols:

1. SHORT-TERM PROTOCOL

Goals:

- a) verify the efficacy of the treatment;
- b) verify the toxicity of the treatment on the honey bees (adopting the Liebefeld method to estimate strength of the hives).

Mite fall counts will be realized on bottom board every 2-3 days.

To verify the survivals of varroa after formic acid treatments, you can use oxalic acid¹ after confinement of the queen in cages for 25 days (Fig. 6), or another treatment realized with 2 different chemicals administered (e.g. 2 strips of Apistan or CheckMite and 2 strips of Apivar in the centre of the colonies, three combs apart, in cross)

To calculate the percentage of acaricide efficacy (*AE*) of formic acid in each hive, use the

following formula:
$$AE = \frac{V_T}{V_{(T+Follow-up)}} * 100$$
, where V_T is the total number of mites killed with

the formic acid treatment and $V_{(T+Follow-up)}$ represents the total number of mites killed by the tested treatment and the oxalic acid or the chemicals.

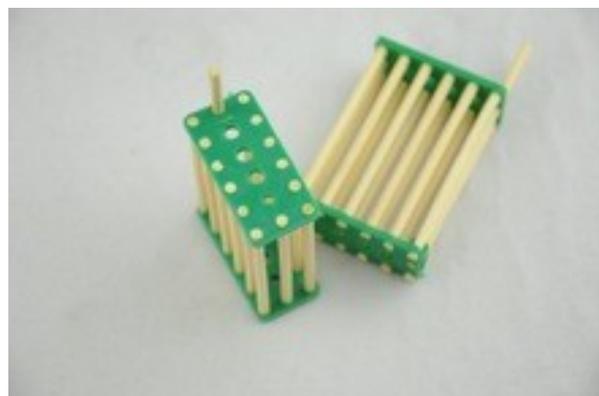


Fig. 6: Examples of queen cages. Var Control -API.MO.BRU (left); bamboo wooden queen cages(right)

Links for cages:

<http://www.apimobru.com/en/ppe/ppe.htm>

<http://www.aliexpress.com/w/wholesale-queen-cages.html>

If possible, at the beginning of the trial record the queen age. Record the queen mortality at the end of the formic acid treatment.

¹

Api-Bioxal according to label or bihydrate oxalic acid, sucrose and demineralized water 1:10:10 in weight. ATTENTION: the use of Oxuvar or Ecoxal leads to insufficient concentration.

Example of “short term protocol”

Each apiary: 8 hives (8 hives/group; 3 groups). For a total of 24 hives

When:

Summer

Experimental groups: (8 hives/group)

1) Nassenheider Evaporator Professional (considering 10 days of treatment).

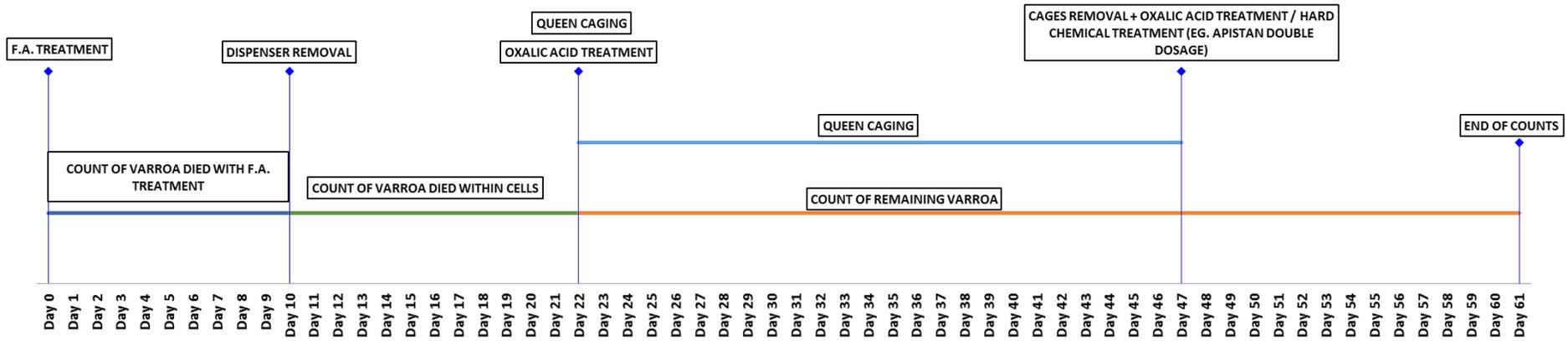
2) MAQS. (considering 7 days of treatment, according to label indications).

3) Untreated (control)

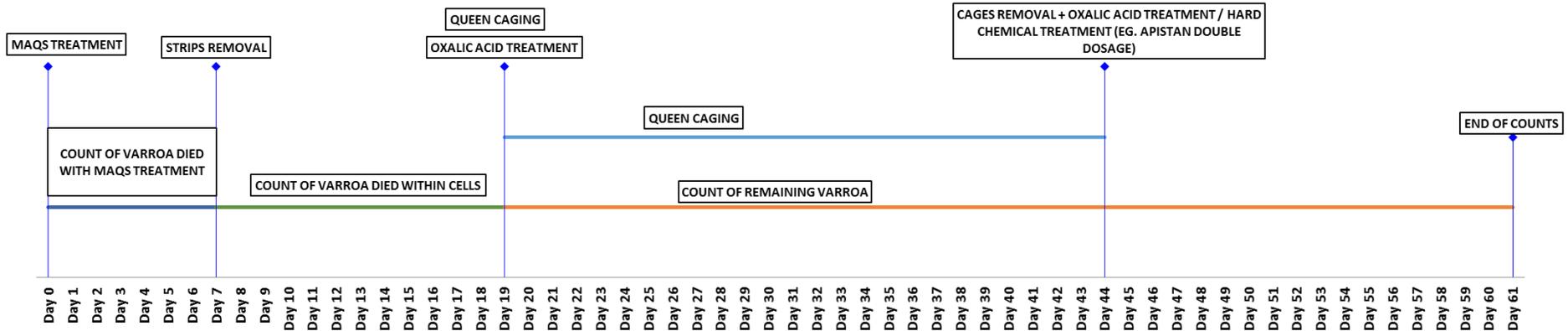
In the Figure queen is caged for less than 25 days as described above: from day 22 to 45 and 19 to 42 and

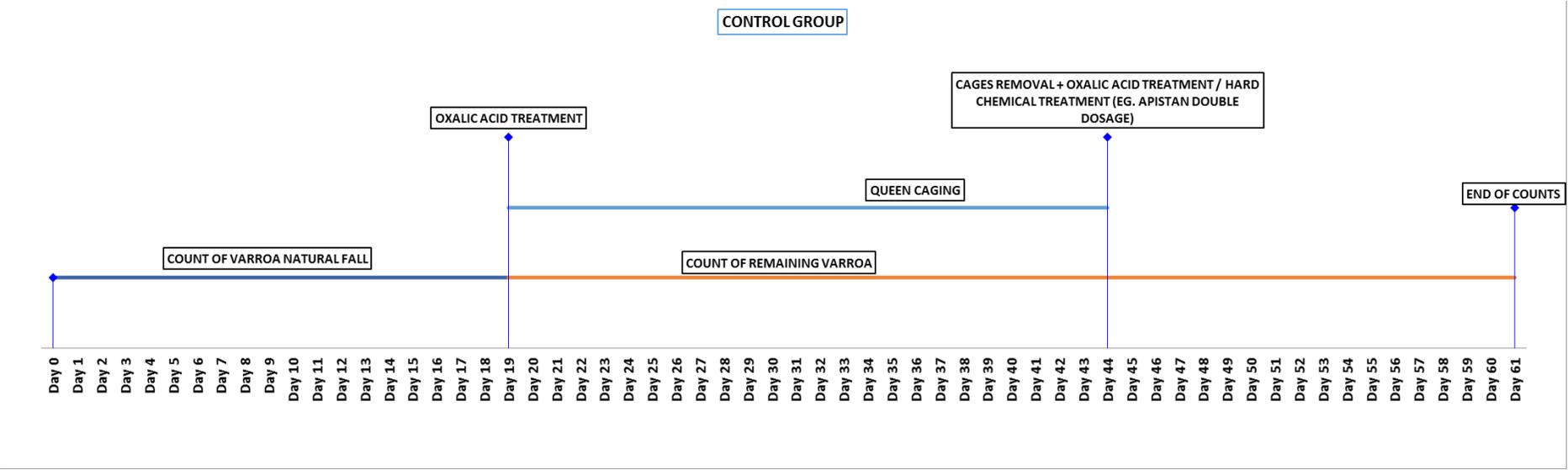
I think that for experiments, chemicals (Apistan, ChecMite...) can be used to perform final treatment, after formic acid application. In this case more accurate data will be obtained to calculate formic acid efficacy.

NASSENHEIDER DISPENSER TREATMENT GROUP



MAQS TREATMENT GROUP





2. LONG-TERM PROTOCOL

To verify: a) the toxicity of the treatment for the honey bees (using the Liebefeld methods) and b) the efficacy of the treatment checking the varroa survivals in Autumn/Winter time. In this case there are more variables: e.g. seasonal variability) and should be used more apiaries. Control treatments: oxalic acid trickling in absence of brood generally November-December in central Europe climate conditions. If you are in an area with no brood stop in Winter, queen caging with your usual method is recommended for an oxalic treatment in November- December. Varroa infestation level: To measure Varroa treatment fall and natural mite fall we will count the Varroa on bottom board every 2-3 days. To protect against ants, we will use paper towels impregnated with oil (canola or sunflower oil) to be changed after each counting.

Between last formic acid treatment and the control oxalic acid treatment Varroa should be counted once a week (if not possible at least twice a month) to monitor any Varroa reinvasion. Flight entrance should be reduced, any feeding with sugar syrup during day prohibited in order to reduce risk of robbery.

Liebefelder method: To measure colony strength and development. Just before first treatment, after the treatment, before wintering (in Switzerland mid-October), and in spring (in Switzerland end of March beginning of April).

Here is the method described:

<http://www.agroscope.admin.ch/imkerei/00000/00294/02107/index.html?lang=en>

You can do the online exercise to train yourself.

In the field for the population measurement by taking a computer with excel (Excel files are provided in separate files) or by printing the population measurement sheet (provided separately in DOC format). You can enter the data then back to the computer.

Collection of the data

All the collected data will be gathered in a common excel file. Like that we all have the data in the same format and ready to use for statistics. This data are the population measurement data, T° and H°, Varroa and the remarks.

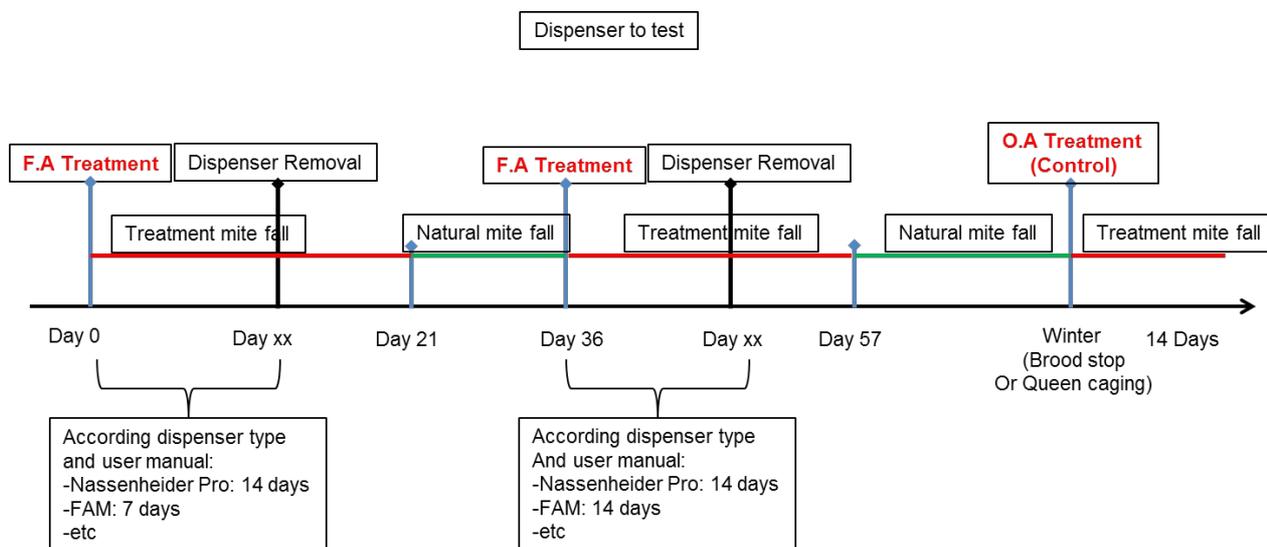
When to start?

Beekeeping season 2014-2015

Weather forecast: by Stefan. This is planned in a later stage.

Example of “Long term protocol”

Time table plan



References

Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees.; European Medicines Agency - EMA (2010)

Available online (last accessed 03.09.2014):

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/11/WC500099137.pdf

Imdorf A, Charrière JD, Kilchenmann V, Bogdanov S, Fluri P (2003) Alternative strategy in central Europe for the control of *Varroa destructor* in honey bee colonies. *Apiacta* 38: 258-278.

Technical guidelines for the evaluation of treatments for control of varroa mites in honey bee colonies; European working Group for Integrated Varroa Control.

Available online (last accessed 03.09.2014):

<http://www.agroscope.admin.ch/imkerei/00316/00329/04435/index.html?lang=en>

MODIFICATIONS: Please write here any modifications of the protocol you had to do during experiment to adapt to your local conditions and local dispensers and why you did it.