Technical guidelines for the evaluation of treatments for control of varroa mites in honey bee colonies

Recommendations from the CA3686

Document prepared during discussions within the CA3686 working group

"Evaluation of treatments for control of varroa mites in honey bee colonies"

I. Standards for experimental protocols

1. General considerations

1.1 The trials should evaluate both the efficacy of the treatment and the direct tolerance by bees. The long term effect from treatment on colony development should also be evaluated.

1.2. Because details can vary for different treatments and under different situations, only general guidelines can be given here.

1.3. It should be taken into account that the evaluation of the efficacy of a treatment is partly conventional and subjected to several causes of error that must be minimized but cannot be avoided completely. Techniques to reduce errors often require contrasting actions: if the protocol is optimized for some aspect, the effect of other sources of error inevitably increases; for example, delaying the control treatments to take into account retarded effects of the treatment increases the risk that the results are influenced by reinfestation.

1.4. Here we indicate the main causes of error and possible ways of overcoming these difficulties rather than indicating a rigid protocol to be adhered to. Unfortunately this approach does not make it possible to define some parameters quantitatively once and for all.

2. Evaluation of the efficacy.

2.1. Hives with a sticky sheet, protected with a net, where the distance between sheet and net is more than 0.8 cm, should be used. The net should cover the whole bottom of the hive for safe collection of mites. Ensure that the net is not obstructed by propolis.

2.2. After the treatment the number of mites fallen onto the bottom is counted at regular intervals. How often and how long counts of mites should be made, depends on the mode of action of the acaricide studied; in principle, mortality rate in treated colonies has to be

compared with that in untreated controls, and appropriate corrections (cf. Abbott's formula) may be required. In case of organic acids the mite fall has to be measured up to two weeks after the end of treatment. This makes it possible to include retarded effects of the treatment but at the same time increases the risk of reinfestation.

2.3. After the treatment, further treatment(s) ("control treatment") are carried out to kill surviving mites (2. 4b) and residual number of mites counted.

2.4. Efficacy is calculated as: (mites killed by the treatment)/(total mites killed by treatment plus control treatment). If the time between the treatment period and control treatment allows a period of natural mite mortality the size of this mortality should be accounted for and excluded from the calculations. Tables 1 and 2 indicate recommended periods for evaluating the treatment effects of certain treatments and control treatments, as well as recommended period between these treatments.

Table 1. Examples indicating recommended periods to evaluate the mite mortality when treatments with ethereal oils and organic acids are applied. FA = formic acid; OA = oxalic acid

Treatment substance	Number of treatments	Period during which falling mites should be counted	Control substance
FA, short term	4-6	Up to the next treatment or 14 days	Perizin or OA
FA, long term	1 or 2	Treatment period + 14 days	Perizin or OA
OA, spraying	1, broodless colonies	14days	Perizin
OA, trickling	1, broodless colonies	14-20 days	Perizin
Thymol	2	Treatment period (4-6 weeks) + 14 days	Perizin or OA

Table 2. Examples indicating recommended periods to evaluate the mite mortality when different control treatments are applied. The control treatment should be applied at the end of the recommended period for evaluating the mite fall due to the treatment being tested.

Control substance	Condition of the colonies	Number of treatments	Period during which falling mites should be counted
Perizin	broodless	1	7 days
Oxalic acid, spraying	broodless	1	14 days

To reduce the error in the assumption that the control treatment is 100% effective, the following should be considered:

a) the ”control treatment” should be applied in the absence of capped brood (exception: substances with a long lasting action such as pyrethroids, if they are still effective)

b) the "control treatment" must have documented efficacy of at least 90-95%

c) acaricides used for the ”control treatment” should not be chemically related to acaricides used for the treatment

d) it must be recorded if mites may reproduce during treatment (i.e. extent of brood rearing)

e) mite mortality other than from treatment should be avoided (In particular, combs and comb foundations contaminated with levels of acaricides which have toxic effects on mites should not be used)

f) there should be no empty comb or other barriers below the bee cluster to assure that dead mites fall directly to the bottom

It should be taken into account that the error due to natural mortality increases rapidly when the efficacy decreases and corrections required may be not negligible under some circumstances (Tab. 3). This should be taken into account especially when evaluating the efficacy of long lasting treatments.

2.5 To reduce the error from infestation of mites between colonies from both outside

Tab. 3 Correction to be made to take into account natural mortality, according to the Abbott’s formula.

	Mortality in treated colonies (%)				
Natural mortality (%)	60	70	80	90	95
5	-2.1	-1.6	-1.1	-0.5	-0.3
10	-4.4	-3.3	-2.2	-1.1	-0.6
20	-10.0	-7.5	-5.0	-2.5	-1.3
30	-17.1	-12.9	-8.6	-4.3	-2.1

sources and within apiary, the following should be considered:

a) trials should be carried out in isolated apiaries whenever possible; if feral colonies are discovered near the apiary they should be removed or destroyed

b) hives should be placed so that drifting of foragers is reduced

c) situations leading to robbing (such as: weak colonies) should be avoided

d) the infestation levels should not endanger or weaken the colonies seriously and cause the loss of many bees (infestation less than 5000 mites in a strong colony)

e) Only colonies where the inspection of debris indicates mite populations between 100 to 5000 mites should be used. An approximate evaluation of the infestation level is obtained by counting the fallen adult mites during 7-14 days on the bottom before treatments (”natural mortality”: 1 mite per day on bottom boards indicates 120-130 mites in the colony in colonies during summer when brood is hatching).

2.6 Other causes of error should be excluded: predation of dead varroa mites by ants or other scavengers should be avoided.

3. Effects on bees

3.1. Hives should be provided with dead bee traps to collect dead bees. Dead bee traps should be emptied with short intervals, if possible daily[1], in particular in connection with treatment. Bottom boards should also be checked for dead bees.

3.2. Bee mortality should be monitored for 7 - 14 days before the treatment, during the whole treatment, and 7 - 14 days after the treatment. Colonies with an abnormally high mortality should not be included in the trial. Colonies should not be remarkably affected by diseases other than varroosis.

3.3. A group of control colonies, with approximately the same number of colonies as one of the treated group(s), is kept at appropriate distance from the treated colonies, or when bee houses are used, placed to minimize drifting. The control group is left untreated or is treated with a standard treatment (the best treatment available), whose side effects on bees under the condition of the trial are well known and reliably constant (*e.x.* fluvalinate where effective, spraying of oxalic acid or trickling coumaphos where effective).

3.4. Colony strength (bees, open and capped brood) is evaluated at 3 weeks intervals during the whole trial period (Liebefeld method). Evaluation should be carried out when flight activity of bees is negligible, for example early in the morning, under the same conditions for control and treated hives.

3.5. The colonies are inspected to note their conditions and the presence of the queen at least at the beginning and at the end of the treatment.

3.6 Long term effects should be evaluated; in particular, under conditions where treatment effect on winter survival is important, estimates of bee strength and brood development should be recorded at least once (better three times) in both treated and control colonies during the spring following an autumn treatment.

4. Other conditions that can affect the results

4.1 The following should be noted:

a) the type of the hive used in an experiment
b) the history of previous treatments in the colonies; if possible, analysis of the combs and comb foundations used should be carried out
c) availability of nectar sources (or other or food sources)
d) flight activity of the bees during the experiment
e) the presence of brood at different periods of the experiment.

4.2. The results of treatments with natural products are influenced by environmental conditions. The temperature in or near the apiary should always be recorded. Recording temperature in the hive interiors as well relative humidity and solar radiation could be useful for research purposes.

II. *Minimum requirements for a practice to be recommended to the beekeepers*

5. Replicating the experiment

5.1 Confidence in a given treatment depends much more on the fact that it has been tested repeatedly under different environmental conditions than on carefully planned methods or refined statistical procedures used to obtain and to analyze the results.

5.2. This is particularly true of varroacide treatments with natural substances, which tend to be influenced by many, partly uncontrolled, factors. A definite weakness of these treatments is the lack of a *standard mode of use* (dose, concentration, duration of the treatment, evaporating pad, etc.). This inadequacy makes the standardization of the methods outlined above difficult. Reliable information on a treatment requires *replicated*, independent tests in a variety of environmental situations.

5.3. The introduction of a new treatment could go through the following steps:

a) Test different variations of the main parameters of the treatment (e.g. concentration and amount of the active substance) on groups of about 10 colonies (or more). Report both positive and negative results; information on negative results is even more important than that on positive results. Preliminary results should not be published in bee journals.
b) Identify the combinations that produce good results; test the most promising again, under a range of different conditions. Tests should be carried out during two different years on a total of (at least) 100 colonies per experimental group. Report

both positive and negative results and try to correlate them with environmental conditions.

c) Describe clearly but concisely the combination(s) of parameters that produce the best results as the *standard* technique, and identify a range of conditions under which this technique produces good results. In this way a standard, *clearly defined* treatment should be identified.

d) Once a standard technique is identified, further adjustments and changes should be compared with this technique in repeated tests before reliable conclusions are drawn.

If intermediate results are published in beekeeping journals, readers should be warned that these results require further tests before they are introduced into use.

6. Residue problems

The safety aspect should never be neglected. The problem has two aspects: the safety for the beekeeper and the quality of honey, i.e. the safety for the consumer.

The mode of application of the product should not endanger the beekeeper; possible risks should be analyzed and appropriate measures should be indicated to avoid injuries even in the case of an accident during the use of the product.

On the other hand, residue analysis should always be part of a scheme before methods are advocated. Samples should be collected according to the following general criteria.

6.1 Sampling frequence and period

It must be taken into account that some varroacide substances (in particular, organic acids and thymol) are natural components of honey. Thymol occurs in lime honey up to a concentration of 0.16 mg/kg. The situation with organic acids is more complex, since the natural content depends on the origin of the honey. For example, honeydew honey may have 2-4 times higher contents of oxalic acid than blossom honey. In order to assure representative results, honey samples from the treated and control colonies must have the same botanical origin; a quick sensorial analysis and conductivity measurements should be carried out for this purpose.

Samples should be taken from the colonies

a) before the treatment

b) after the end of the last treatment

c) the following spring, if the treatment is carried out in the autumn

d) from the super at the next nectar flow

6.2 Combs from the nest

Sampling sites within the brood nest should be representative.

a) Samples should be collect from three different points (external frame front bottom corner, external frame rear upper corner, middle frame middle upper or bottom position)

b) if a dispenser was used (e.g. strip, evaporating pad, modified frame, etc.) the sampling site should be chosen near, mid and far from this device

c) if evaporating substances were used, it should be recorded if samples originate from capped or open honey

6.3 Combs from super

Sampling site should be representative for the supers. The suggestions given above, especially a) and c), should be considered.

6.4 Extracted honey

If extracted honey is analyzed, it is recommended that a representative sample from a lot is taken by chosing honey on the bottom, middle and top of a tank or ripener.

6.5 Wax

When lipophilic active ingredients are used it is recommended to sample also nest comb wax according to the same scheme applied for honey. At least 10 g of wax is required for the analysis.

6.6 Other conditions that can affect the results

The following should be noted

a) quantity of honey at the moment of the treatment

b) total amount of extracted honey

c) nectar flow in the period considered.

6.7. Other contaminants

Attention should also be paid to the possible presence of impurities, secondary reaction products, coformulants, stabilizers, etc. in the products used for treatment. If these substances are of any toxicological importance, their residues should be determined. Special attention should be paid to breakdown products and products that may arise from the reaction with substances present in the hive.

III. The evaluation of concepts of integrated Varroa control

From experience we know that different treatments need to be combined to keep the Varroa population below the damage threshold. Such control concepts vary depending on climate, foraging conditions and beekeeping management. Therefore such concepts have to be evaluated locally before recommendations should be made to beekeepers.

To evaluate the efficacy of a concept for integrated Varroa control, control treatments can not be applied. It is instead necessary to estimate the fluctuations of the Varroa population by monitoring the natural mite mortality at specific times of the year, depending on the concept and other conditions. The damage threshold, based on the observation of the natural mite mortality, should be defined in relation to the condition of the area considered; under most circumstance, in Central Europe it can be set to never exceed 20 to 30 mites per day in bottom debris.

The concept ot integrated control should be tested for at least two years, preferably three or more.

Tab. 4. Summary table for standards for experimental protocols for the evaluation of treatments against Varroa

Treatment efficacy
Use hives with net bottoms and boards for mite counts
Use colonies with infestation of 100 - 5000 mites in normal colonies
Count mite mortality before, during and after both treatment and control treatment
Efficacy is calculated as: $\frac{\textit{mites killed by the treatment}}{\textit{mites killed by treatment} + \textit{mites killed by control treatment}}$
If possible control treatment - should not be applied in colonies with sealed brood (unless the acaricide is suitable for this) - should be repeated until mite mortality is negligible) -should be at least 90% effective - should not be chemically related to treatment acaricide
Presence of brood during treatment should always be recorded
Wax used in experimental colonies should not contain acaricide residues at levels that can affect mite vitality
Reinfestation of mites during an experiment should be minimized

Bee tolerability
Both treated and control colonies should be equipped with dead bee traps
Bee mortality should be monitored before, during and after treatment
Control colonies should be left untreated or treated with an acaricide with a known and reliable effect on the bees
Colony strength (bees as well as open and sealed brood) should be recorded at intervals of three weeks during the whole trial period
Where appropriate, early colony development in the season following the treatment should be recorded in all colonies
The presence of the queen before and after the treatment should be recorded