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# "Coordination in Europe of integrated control of *Varroa* mites in honey bee colonies"

**APPENDIX VI**

to

Final Technical Report for the period

from **98-01-01** to **99-12-31**

# Final Technical Report

TABLE OF CONTENTS Page

The Commission Of the European Communities, Director General for Agriculture (DG VI.F.II.3) has given support 1998-1999 for a Concerted Action 3686, entitled “Coordination in Europe of research on integrated control of *Varroa* mites in honey bee colonies”

BACKGROUND

Varroa mites in European honey bee colonies must be controlled if colonies are to survive. The most common treatment regimes involve use of synthetic acaricides inside the bee colonies. Although effective, these control methods have created new problems for the beekeeping in Europe, such as acaricide residues in bee products and, lately, acaricide resistant mites.

Many studies demonstrate that several organic acids, already present in small amounts in bee hives (lactic, formic, oxalic acid) can be used to control varroa mites. There is also growing evidence that some etheric oils and some common food additives, as well as biotechnical management, can be used effectively for mite control. However, these ecological alternatives to synthetic acaricides are generally more laborious to use and show variation in efficacy depending on external factors and colony condition. Thus, they need more attention and knowledge on behalf of the beekeeper to be sufficiently effective. From practice it is also evident that methods that work well in one region may be inappropriate in another with a different climate.

Thus, there is a large need for a network of scientists in this field, capable of identifying the research needs, to harmonize efficacy evaluation protocols, and disseminating research results and recommendations in a comprehensive form that may be used as guidelines for beekeepers in Europe.

OBJECTIVES

The main objective of this Concerted Action (CA) is to coordinate research efforts in Europe on integrated varroa control and to disseminate information to the beekeeping practice on how pesticide use in beekeeping is best avoided.

Specific objectives of this CA are to:

* Establish a European network of scientists for efficient exchange of information on methods that minimize the use of pesticides for control of the parasitic mite *Varroa jacobsoni* in honey bee colonies.
* To harmonize efficacy evaluation methods.
* To highlight future research needs in this field and to initiate new collaborative research.
* To compile information about integrated control of Varroa in a format suitable both for publication in national beekeeping journals and as a separate publication, and to initiate seminars on this subject.
* To make available a bibliography on ecological control of *Varroa* mites.

Texts in this compendium are working material presented and discussed by invited European scientists, advisors and legislators at the CA meeting at the Agricultural Research Centre-Ghent, Belgium, November 13-14, 1999.

Uppsala, 1999-10-28
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# Building Strategies for Varroa control

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## Summary

During the first years of the appearance of the varroa mite it was supposed that the mite mainly damages the adult bees. But adult bees, however, succeed quite well in spite of being mite infested. The initial damage by the varroa mite takes place in the brood. One reproducing varroa mite in the brood cell is enough to severely influence the future life of the emerging bee. From a brood cell parasited by several mites fully developed bees cannot emerge. The clinical symptoms of dying brood resemble those of European foulbrood. Virus infection can worsen the damaging effect of the varroa mites.

During the season there is a steady increase in infestation rate of both brood and adults, and consequently, in the number of damaged bees. Varroa control exclusively by chemical preparations can only be successful at low infestations or if treatment early in the season is possible. It is necessary to take measures against the varroa during the whole year. This includes the cutting of drone brood, forming of nucleus and chemical treatments. In the middle and at the end of the season it is necessary to control the natural mite mortality. The number of mites in the debris gives an indication of the urgency of chemical treatments.

## Introduction

Under normal conditions, controlling parasites is not problematic. Generally, parasites do not kill their hosts because this would also endanger their own existence. From an evolutionary standpoint, the host-parasite-relations between varroa and the Western honey bee is not yet established because the transfer to the new host from the Asian honey bee took place quite recently.

For parasites that do endanger host survival control strategies are necessary. For the development of a control strategy it is of essential importance where, when and how the parasite damages its host. This paper comments on how strategies for varroa mite control in honey bee colonies should be developed.

## Varroa infestations and bee damages

During the first years of the appearance of the varroa mite it was supposed that the mite mainly damages the adult bees. But adult bees actually succeed quite well in spite of being mite infested. The loss of haemolymph (bee blood) is limited. At each sucking the mite hurts the pellicle between the abdominal rings (intersegmental membrane). As the mite can only absorb small quantities it hurts the bee several times within a comparatively short period, thus provoking the invasion of disease agents (fungi and bacteria). Moreover, viruses get into the bee corps via the saliva of the mite. Partly because of a well functioning endogenous immune defense system, the mite infestation and the loss of blood caused by a parasitation of up to two mites does not cause acute danger for the adult bee. Only if several mites attack the bee at the same time severe damages can be observed. Such a severe infestation of individual adult bees, however, only occurs close to colony collapse when mite populations have grown large enough to damage both brood and adults.

The initial damage by the mite takes place in the brood. One reproducing varroa mite in the brood cell is enough to severely influence the future life of the emerging bee. The life span of bees parasitised as pupae is shortened. Activities like hive hygiene, processing of food reserves and guarding services are also affected negatively. Parasitised brood produces bees that are not fully integrated as a productive member in the system of work division, which is of essential importance to the bee colony. Badly developed brood-food glands (hypopharyngeal glands) impede a long-term activity as nurse bee and the bee becomes a foraging bee at an earlier age.

From a brood cell parasitised by several mites fully developed bees cannot emerge. When the damage is visible, their life span is shortened to such an extent that they die already within a few days. This effect is, of course, even more pronounced for brood parasitised by a larger number of mites. Such bees often show deformations of wings and of the abdomen. Severe infestations of the brood often result in mortality already within the cell. The clinical symptoms of dying brood resemble those of European foulbrood. Especially the paralysis viruses (Acute Paralysis Virus and Slow Paralysis Virus) can worsen the damaging effect of the varroa mites. Viruses can provoke changes in bee behaviour similar to those caused by varroa mites. The life span of bees into which viruses are injected during pupae-stage in their development is shortened and such bees only work as nurse bees for a short time. With severe virus infections the brood dies showing symptoms of European Foulbrood. Deformations of the wings and the abdomen may also be caused by the deformed wing virus (DWV). As this virus only causes severe symptoms in a colony simultaneously infested by mites it was not considered important for practice before the introduction of varroa mites. It is generally valid that viruses strengthen the damaging effect of mites.

In certain years, viruses only appear in rare cases for reasons unknown up to now. When virus infections associated with the mite are not prevalent, bee colonies can survive populations of several thousand mites without problems. If, however, mites and viruses attack the bees, smaller number of mites may be enough to lead to colony collapse. Therefore, it is useful to recognize when an infestation by mites increases as early as possible and if there is a simultaneous virus infection. The mite infestation level can easily be observed by the beekeeper, the virus detection needs the help of a laboratory.

## Mite population growth and strategies for prevention

The mite reproduction possibilities increases with the amount of brood rearing and the duration of the brood rearing period. But also the bee density in a region and the subsequent pressure of infestation from colonies where varroa mite populations are not controlled finally decide the needs for mite control and the number of mites in a colony. Since the mite mortality, including the winter period, does not outweigh the mite reproduction capacity in any European climate where bees occur, the number of mites in the bee colony increases if left uncontrolled.

Under average conditions, in Central Europe, the mite infestation increases during the second half of the year. The more the number of brood decreases the more the number of brood cells infested by the mites increases. Thus, there is a steadily increasing number of damaged bees. In August/September the winter bees are reared. Only if the infestation of this brood is low the colony will manage to winter well and expand the following spring. Varroa control measures in July/August serve the need of getting fully developed winter bees. If varroa control is made only in September or later the proportion of damaged winter bees depends on the number of infested brood cells. If the treatment is late, many winter bees may be damaged and colony survival over winter will decrease.

Consequently, the following principle is valid: Every control measure of mites done before July/August is for the benefit of the winter bees and a later treatment helps the summer bees the following year.

Varroa control exclusively by chemical preparations can only be successful at low infestations or if treatment is possible early in the season. Chemical preparations usually produce residues in bee products. They should not be used before and during honey production. In highly infested colonies the bees are already severely damaged by the mites and the viruses, before a first chemical treatment is possible. In some years, even preparations with 100% effectiveness are not sufficient to save the colonies because the damages caused by the virus infections are not removed by removing the mites.

## Varroa control during the whole year.

As a rule varroa control can be divided into three phases:

1) At the beginning of the season measures have to be taken to secure that the bee colonies enter the following nectar flow phase with the lowest infestation possible. As the application of chemical preparations for control immediately before a foraging period is not recommended, biotechnical methods, such as cutting out of drone brood and forming of nuclei, have to be used.

2) In the summer, the natural mite mortality is investigated in colonies equipped with net screen bottoms. The number of mites in the debris indicates the urgency of chemical treatments.

3) In the late season it is vital to control the natural mite mortality again. This is true for all methods of treatment irrespective of how effective they may be. Through reinvasion from collapsing colonies many mites may have penetrated into the colonies already treated or the chemical used for control may not be effective any longer because the mites may have developed resistance to the chemical used. The success of treatments should also be verified at this time. This is especially important if new method have been applied for the first time or if decreased efficacy of the preparation can be suspected due to resistance of the mites.

The control concepts can be adapted in many ways to the management method used, the hive system, and the requirements of the beekeeper. This is valid for the biotechnical methods as well as for the chemical alternatives. The method of cutting out drone brood depends very much on the hive type and the rhythm of operating the hives. Also the forming of nuclei can be made in different ways and at different times depending on the season and the general beekeeping system used. Chemical preparations are chosen mainly on the basis of their efficacy, where efficacy also in the sealed brood cells should be considered. Preparations not effective in sealed brood cells either require long exposure in the colonies, or have to be used in brood less colonies to be effective. So far, only formic acid treatments have been shown to be effective on mites in sealed brood cells. Another important factor for selection of chemicals for mite treatment is the danger of residues. Of course, only products registered for use in bee hives should be used for mite control and the label instructions should always be followed carefully.

At last, the hive system and the management method are again important criteria for what methods of mite control that are most convenient to adopt. Also personal preferences and general attitudes regarding chemicals and residues in bee hive products will be important criteria for individual choices of mite control strategies. For the practice it should be noted that it is not which chemical that is used which decides final success in mite control, but how a complete treatment concept is applied and adapted to local circumstances.

# Oxalic Acid for Mite Control - Results and Review

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## Summary

*Optimisation of the trickling method*

In order to make oxalic acid (OA) use against varroa mites easy and quick, a new administration technique was set up in 1996. According to this method an OA acidified sugar syrup (dehydrated OA, sucrose and water in ratio (weight) 1:10:10) is trickled by a syringe onto the top bars and in the space between combs with a dose of 5 ml/Dadant-Blatt comb populated by the bees. However, since OA and sucrose concentrations and doses were fixed empirically, some research to optimise this method was needed.

Specific trials were carried in various European countries (Finland, Germany, Italy, Norway, Sweden and Switzerland) to test different combinations between OA (0%, 2,1%, 3,2% and 4,2%) and sucrose (0%, 30%, 60% and 70%) concentrations. It was clear that sugar solutions containing 4,2% OA is the most effective: when administered according to the protocol it yielded an average mite mortality ranging between 90,3% and 97,8%. Nonetheless, in more limited trials the 3,2% option gave similar results. According to these trials, 2,1% OA concentration do not yield sufficient mite mortality for normal mite infestations.

Sucrose seems to be needed in the solution, since non-sugar options usually gave poor effectiveness. However, 30% sugar concentration could be enough since little (if any) differences were detected in comparison to 60% options for a given OA concentration.

With few exceptions, bee mortality at the hive entrances was normal after OA administration. No increase in winter mortality of treated colonies could be clearly related to the treatments. However, in mid-European trials some OA concentrations have demonstrated colony weakening as a result of OA treatments with an effect also registered the following spring. These negative effects seemed to disappear at 2,1% OA concentration. Italian observations, that need to be further confirmed, also indicate some negative effect on spring build up of colonies when 4,2% OA is used in high sugar concentrations (60% and 70%).

In The Netherlands, the effect of variations in the trickled amount was tested. A solution having 3,6% OA and 60% sucrose concentrations was used; one group of colonies received

2,9 ml/populated comb (corresponds to the same volume/surface ratio of 5 ml/Dadant-Blatt frame for the Dutch frame) and another one only 2,5 ml. The larger volume was more effective (92%), but was less well tolerated by the bees.

*Tests on OA toxicity for the bees*

From colonies belonging to an OA treated and an untreated group, pupae were collected before administration of OA by trickling. Fifteen days after the treatment, newly emerged adults and pupae were sampled again from the same colonies. The Glutathione S-transferase (GST) activity was determined in the laboratory in each sample. Statistical comparisons could not demonstrate any significant difference in GST activity related to treatments. According to these findings it seems that an OA trickling at normal dose does not compromise the bee digestive system or weaken the detoxifying activity against potentially harmful substances.

*Study on the mode of action of OA*

In two experiments applying OA solutions by trickling and spraying were compared to the corresponding solutions based on potassium oxalate for their acaricide effect. The solutions compared in each trial differed in their pH, being very acid (OA) or neutral (potassium oxalate). The mite mortality was very low in the groups receiving the neutral solutions and much higher in the acid treated groups.

From these experiments, it seems that the acidity is responsible for the OA activity against the mites and not the oxalate ions in the solution; however the sensitivity to the acidity still remains unexplained.

## Introduction

The effect from oxalic acid (OA) on varroa mites has been known for several years. Past experiments have included various administration methods toTholonies: vaporisation (Popov et al., 1989); fumigation (with formic acid development during heating) (Okada & Nekane, 1987); spraying weak solutions onto adult bees (Radetzki et al., 1994; Nanetti et al.; Imdorf et al., 1995), and trickling OA acidified sugar syrups into the colonies (Nanetti & Stradi, 1997). Some of these techniques showed a very high efficacy, although different experimental conditions and methods make direct comparisons difficult

In some European countries spraying bees in broodless colonies with a 2-3% OA in water solution is commonly used amongst beekeepers. This technique is very effective but, since every comb of bees has to be sprayed on both sides, it is usually considered too labour-intensive and time-consuming (and therefore uneconomical) by large-scale beekeepers.

Attempts to make application easier led to a new administration technique being developed. An OA acidified sugar syrup made up of dihydrated OA, sucrose and distilled water, 1:10:10 by weight, (OA concentration 4,2%, sucrose concentration 60%) is trickled by syringe into broodless colonies. A 5 e ml dose for each Dadant-Blatt comb (dm (3,00*4,35) = dm2 13,05/side) occupied by adult bees is used. This is eqivalent to 0.38 ml solution per square dm covered by bees. Trials in Italy showed a high acaricidal activity (96,8% and 96,1% in 1996 and 1997 respectively) without any ill effects in test colonies, even at very low temperatures (Nanetti & Stradi, 1996 and unpublished data). In 1996 an efficacy of 89,6% was recorded using a solution with the same sugar strength but an OA concentration of 2,2%. On the basis of these results, and on the results of trials performed by beekeeper organisations, many Italian hobbyist and professional beekeepers now use this method in autumn treatments against varroosis.

However, in some instances, bees show poor tolerability to OA trickled into the hive. Colonies receiving an overdose (i.e., excessive amounts, short-term repeated administrations, or excessive OA concentrations) can be weak at the end of the winter or sometimes fail to over-winter at all. In these cases no abnormal bee mortality is usually seen at the hive entrance. However, according to both published (Charrière et al., 1998) and unpublished data, some trials have also found that bees do not tolerate the trickling treatment very well at normal dosages.

An OA specialist team from within the CA3686 group developed a series of experiments to improve our understanding of the treatment, with special reference to the trickling technique. The findings so far are summarised below, together with the results of independent, but related, experiments by researchers belonging to the group.

## Optimisation of the trickling solution

Sugar and OA concentrations for the solution used in Italy were chosen empirically. This experiment aimed to answer the following questions:

- can better results be obtained using different OA and/or sucrose concentrations?
- is sucrose important for the acaricidal activity or tolerability?

A two-stage experiment was set up. Nine solutions were screened (i.e., every combination of 0%, 2,1% and 4,2% OA concentration and 0%, 30% and 60% sugar concentration) in Nordic countries, where early brood interruption is expected. The most effective combinations were then tested in Southern European areas, where the brood rearing season is longer. However, some extra solutions not included in the Nordic tests were also tested at the second stage. This procedure allowed both stages to be conducted in the same autumn-winter period.

The dose administered varied according to colony strength and, in most cases, to comb size. It was trickled into the gap between two populated combs and onto the respective top bars. Efficacy was calculated as a ratio between the mite mortality due to the treatment and the total infestation level based on control treatments with acaricides. Bee tolerability was assessed by checking mortality outside the hive and by comparing the colony conditions before the treatment, at the end of the winter, and at the beginning of the honey flow. These evaluations were performed by the Liebefeld method.

Table 1 summarises the efficacy recorded in the first stage of the study. Column one of the Finnish results refers to doses calculated on the basis of 100% populated combs; this generally led to under-dosing the treatment and therefore to lower effectiveness.

The highest efficacy was achieved with sugar solutions having the highest OA concentration. The actual values are very close to Italian results. A 2,1% OA solution generally produced a lower efficacy and more variable results.

Solutions without sugar resulted in poor and variable efficacy, even at a 4,2% OA concentration. Also, no clear differences in acaricidal activity were observed between the 30% and 60% sucrose solutions for a given OA concentration, in the same apiary.

Table 2 summarises the average efficacy recorded in the second stage of the study. Again the combination 4,2% OA and 60% sucrose resulted in a remarkably high acaricidal activity. However, the treatments in Italy resulted in a slightly lower effectiveness than that recorded in previous Italian trials which might be due to the low temperatures during the treatment period in this experiment. It is possible that the reduced efficacy in Finnish trials are related to the much lower temperature during treatment in Finland. The effects from temperature during treatments need further studies. Nevertheless, the 30% and 60% sugar solutions for a given OA concentration still yielded similar results and much better efficacy than using water solution. However, in Switzerland also the sugarless solution gave a noticeably high efficacy. The weakest OA solution (2,1%) resulted in less than 90% efficacy, but 3,2% OA approached the effect of the strongest one.

Although the results for acaricidal activity were substantially consistent, some important differences in bee tolerability were observed. The end of winter and the spring colony development measurements carried out in Scandinavia did not demonstrate any detrimental effect on the colonies caused by any combination of OA or sugar concentration. However some bee mortality was observed outside hives in the days immediately after the treatment in a few cases. In contrast, evaluations made in Switzerland and Germany showed a tendency to higher bee losses during winter with the 4,2% OA sugar solution. Lower concentrations resulted in a better over-wintering and spring development.

In Italy, when the treatment was administrated in a cold environment (mountainous), sugar concentration (30% and 60%) did not seem to affect either over-wintering or spring development. However, in foggy conditions the spring measurements indicate that there may be some long-term reduction in colony strength when 4.2% OA solution is used with high sugar concentrations (60% and 70%).

The colony losses recorded during winter in the different countries were no higher than would normally be expected. None could be clearly related to treatment.

## Conclusions on solution optimisation

The 4,2% OA solution in 60% sucrose showed a remarkable effectiveness against varroa mites in broodless colonies treated in the autumn/winter using the trickling method. Although in many cases the bees seem to tolerate the treatment well, in some conditions this OA concentration caused poorer over-wintering or a slower spring development of colonies. Short-term mortality was not seen outside the hive.

Tolerability is better with 3,2% OA. According to the mid-European experiments the differences in efficacy of this concentration compared with 4,2% OA is of little consequence for the beekeeper. A 2,1% OA concentration, however, reduces the risks for the colony but its lower efficacy means that it can only be used when the infestation level is rather low.

Sugarless solutions have poor efficacy and are not practicable, but a reduction of sugar concentration from 60% to 30% seems to have no significant influence on acaricidal activity. This also seems to be true for OA concentrations of less than 4,2%. There is also a possibility that a lower sucrose concentration enhances the ability of bees to tolerate the treatment solution, although no conclusive evidence of this emerged from the trials. Further investigation is needed.

The possibility that high environmental humidity levels reduce the bee tolerability should also be further studied.

## Optimisation of the amount trickled in the colony

We need to verify the optimal amount of solution that must be given to the colonies. A trial was conducted in the Netherlands with this in mind. One solution of 3,6% OA in 60% sucrose was used. It was trickled into colonies according to the method described above, but in variable amounts. Some colonies received 2,9 ml/comb (this dose corresponds to 5 ml/Dadant-Blatt comb because Dutch combs are smaller) and other ones were given 2,5 ml/populated comb. A group of untreated colonies was used as a control.

Efficacy was calculated as the ratio of the mite mortality due to the treatment to the total infestation level. Colony strength was evaluated by shaking and weighing the bees before treatment and then again in the spring.

The larger volume was 92% effective, in line with the values found in the previous trials using 3,2% and 4,2% OA sugar solutions. However, the smaller volume resulted in a remarkably lower effectiveness (80%). A reduced bee population was found in both treated and untreated colonies in spring compared to pre-treatment. The colony weakening seemed to be related to the amount of solution given to the colonies. The reduction of the bee population was 72%, 58% and 41% for colonies treated with 2,9 or 2,5 ml/comb (same amount of active ingredient) or in untreated controls respectively. This experiment underlines the need to establish the optimal dose that will be both effective and safe for bees. Further trials are still clearly needed.

## Laboratory tests on bee toxicity of OA acidified sugar syrup

The tissues of the honey bee gut have glutathione S-transferase (GST) activity. This important group of enzymes acts as a detoxifying system against potentially harmful substances with which bees may come into contact. A lowering of GST activity could make bees more vulnerable to environmental toxic substances.

The following experiment (Brødsgaard et al., 1999) was carried out to discover if OA-sugar solution ingestion reduces GST activity in bees:

Pupae were collected from colonies immediately before treatment by OA trickling and from untreated controls. Fifteen days after the treatment newly emerged adults and pupae were taken from the same colonies. The average GST activity in these samples is summarised in table 3.

The statistical tests performed on the data sets for both pupae and adults did not show any significant difference in GST activity attributable to treatment (comparisons between groups and within each group). However the treatments were performed using normal doses. It is still possible that some detrimental effect on GST activity may occur in case of overdosing. More research is needed to fully understand the treatment-related negative effects, with special consideration being given to colony weakening that can result in certain circumstances.

## Study on the mode of action of OA

The pH/concentration curve is much lower for OA solutions than for the majority of organic acids – even at low concentrations OA, the acidity is very high. The theoretical pH values for the solution used for spraying onto bees (2,1%) and for the solution used for trickling (4,2%) are about 1 and 0.9 respectively. Besides, both solutions have a noticeable efficacy against varroa mites.

To understand whether the OA effect on mites is related to the chemical and toxicological features of oxalate ions coming from acid dissociation in water, or to the high acidity of the solution, two experiments were carried out on broodless colonies. In the first experiment the trickling method was used, whereas spraying was used in the second. In each case one group of colonies was treated by an OA solution (acid) and a second group received a potassium oxalate solution (neutral) both having the same molarity. Efficacy was calculated for each colony as the ratio between the mite mortality due to the treatment and the infestation level.

In both experiments the mite mortality was very low in the groups receiving the neutral solution, but in the other groups a very high average efficacy of about 90% was recorded (table 4). It seems that acidity is responsible for the OA action against varroa mites but the reasons for this are not known. The poorer efficacy of other organic acids (e.g., lactic and citric acids) could be due to their lower dissociation constant.

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**TABLE 1. Average effectiveness recorded in the 1st stage of solution optimisation experiment.**

| Apiary | Sugar | OA | FINLAND | | SWEDEN | NORWAY | | |
|---|---|---|---|---|---|---|---|---|
| 1 | *0%* | *0,0%* | 5,5 | | 5.0 | 5,4 | 2,0 | |
| | *0%* | *2,1%* | 39,1 | | 22.3 | 39,2 | 36,7 | |
| | *0%* | *4,2%* | 48,8 | | 50.9 | 86,8 | 75,0 | |
| | *0%* | *1,0%* | | | 11.0 | | | |
| 2 | *30%* | *0,0%* | 5,3 | | 4,4 | 4,1 | 2,0 | |
| | *30%* | *2,1%* | 35,9 | | 39.1 | 62,4 | 63,0 | |
| | *30%* | *4,2%* | 92,9 | | 94,8 | 91,3 | 84,0 | |
| 3 | *60%* | *0,0%* | 8,2 | 14,9 | 4.2 | 9,3 | 28,4 | 2,7 |
| | *60%* | *2,1%* | 10,8 | 81,5 | 68.2 | 85,5 | 82,5 | 32,2 |
| | *60%* | *4,2%* | 79,7 | 93,2 | 96.1 | 96,5 | 95,1 | 93,8 |
| 5 | *0%* | *2,1%* | 5,4 | | | 65,2 | 69,3 | |
| | *30%* | *2,1%* | 6,0 | | | 93,5 | 88,9 | |
| | *60%* | *2,1%* | 15,4 | | | 92,5 | 82,9 | |
| | *0%* | *0,0%* | | | | | 5,3 | |
| | *60%* | *0,0%* | | | | 3,6 | | |
| 6 | *0%* | *4,2%* | 29,0 | | | | | |
| | *30%* | *4,2%* | 76,4 | | | 97,2 | 92,7 | |
| | *60%* | *4,2%* | 83,9 | | | 97,8 | 94,3 | |
| | *60%* | *0,0%* | | | | 7,6 | 4,4 | |

**NOTE**: REDUCED FONT: different method for dose evaluation
ENLARGED FONT: possibly some brood residual in some colonies

**TABLE 2. Average effectiveness recorded in the 2nd stage of solution optimisation experiment.**

| Sugar | OA | SWITZERLAND | GERMANY | ITALY |
|---|---|---|---|---|
| *0%* | *4,2%* | 92,5 | | |
| *30%* | *4,2%* | | | 89,6 |
| *60%* | *0,0%* | 3,5 | 3,4 | 3,2 |
| *60%* | *2,1%* | 86,7 | 84,8 | |
| *60%* | *3,2%* | 98,6 | 92,2 | |
| *60%* | *4,2%* | 97,5 | 94,3 | 90,3 |

**TABLE 3. Average Glutathione S-transferase activity in honey bees before and after OA trickling (Brødsgaard et al., 1999).**

| | Treated group | Control group |
|---|---|---|
| **Pupae (before treatment)** | **194,1** | **184,4** |
| **Pupae (15 days after the treatment)** | **207,0** | **191,5** |
| **Newly emerged adults (15 days after the treatment** | **226,2** | **211,5** |

# Use of Essential Oils for the Control of *Varroa jacobsoni*

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## Summary

Essential oils and essential oil components offer an attractive alternative to synthetic acaricides for the control of *Varroa jacobsoni.* They are generally inexpensive and most pose few health risks. Terpenes (mainly monoterpenes) are the main components of essential oils, comprising about 90% of the total. More than 150 essential oils and components of essential oils have been evaluated in laboratory screening tests. Very few of them, however, have proven successful when tested in field trials. Thymol and thymol blended with essential oils or essential oil components offer a promising exception. Mite mortality obtained with these formulations typically exceeds 90% and often approaches 100%. In addition, residues in honey are low, even after long-term treatments. The exact conditions under which these formulations will yield reliable and effective control, however, have only been determined for certain European regions. Based on the available studies, relying solely on a single treatment with an essential oil or essential oil component is generally not sufficient to maintain mite populations below the economic injury level. Therefore, efforts are necessary to optimise the use of these substances and to incorporate them, along with other measures for limiting mite populations, into an integrated pest management strategy for control of *Varroa jacobsoni*.

## Introduction

Colonies in temperate regions must be treated once or twice a year against *Varroa jacobsoni* to maintain their populations below economic injury levels. During the last 10 years, the pyrethroids have been the primary source of insecticides used to control *V. jacobsoni*. Recently, mites in parts of Europe and North America have developed resistance to pyrethroids. The widespread use of synthetic lipophilic acaricides has lead to the accumulation of residues in beeswax, propolis and to a much lesser degree, in honey.

The development of acaricide resistance in *V. jacobsoni* populations and the spectre of the contamination of hive products provide considerable incentive to develop new treatment strategies that minimise the potential for acaricide resistance and the accumulation of residues. Since *V. jacobsoni* was introduced to Europe, intensive efforts have been made to develop alternative chemical control measures based on formic, lactic and oxalic acids combined with biotechnical measures.

It is well known that many essential oils and their components exhibit acaricidal activity. Before *V. jacobsoni* was a world wide pest, different components of essential oils were tested for their activity against *Acarapis woodi*. Methyl salicylate and menthol proved to be toxic to the tracheal mite. In the last 15 years, research has shown that several essential oils and individual compounds of essential oils also have a high acaricidal activity against *Varroa jacobsoni*.

## The potential of essential oils as varroacides

In extensive screening tests, many oils show significant acaricidal activity. Some oils are repellent to *V. jacobsoni*, others are attractive, and some cause mite mortality. However, of more than 150 essential oils and components of oils tested, only very few have proven effective when applied in hives in field trials. This is most probably due to the fact, that the screenings tests used were incapable of predicting the acaricidal effect under field conditions. Difficulty in obtaining standardised essential oils also affects treatment predictability. Only a combination of wintergreen oil and thermal treatment, an aerosol treatment of a thyme-sage oil mixture, and the passive evaporation of thymol, oregano oil and marjoram oil in combination with diluted formic acid have been used successfully for mite control. For different reasons, however, none of these treatments have been widely adopted by beekeepers, with the exception of thymol. Indeed, thymol and thymol blends are widely used to control *V. jacobsoni* in Europe and in most cases their varroacidal efficacy is greater than 90 % (Fig. 1 and 2). Different thymol containing products are available on the market.

Because of insufficient predicting capacity of the screening tests used until now, we devised an assay, in which the dose-response relationship of an airborne acaricide and the corresponding mite and bee mortalities can be assayed under laboratory conditions. Using this technique, a high mite toxicity, combined with good bee tolerance, were demonstrated, besides thymol, for the following components of essential oils: camphene, camphor, p-cymene, eugenol, isopinocamphon (ysop oil), menthol and $\alpha$-thujone. Identifying compounds with acceptable acaricidal activity but with low toxicity to honey bees is essential for providing candidate compounds for field trials. After finding suitable substances under laboratory conditions we will measure the air concentration under field conditions to test their efficacy in a bee colony. This procedure can serve as a powerful screening technique because it guides subsequent field research into the most productive avenues. The development of effective delivery systems for essential oils remains one of the greatest obstacles to their implementation as mainstream control measures. Highly volatile substances like camphor are difficult to use, but formulations retarding the evaporation rate, e.g. special gels, might overcome this difficulty. Products with mixtures of different components with different modes of action, might also provide effective solutions. For example, substances that disrupt the mite's host finding behaviour may be effective in conjunction with substances that kill mites.

## Residues

Residues pose another challenge to the use of essential oils. Most essential oils are mixtures of more than 50 components. Depending on the individual partition coefficients of the constituents, residues in honey and wax are to be expected. Residues in honey can lead to adverse effects on taste, while residues in wax can render it unsuitable for some applications. Quantitative residue analyses are required for product registration. The complex nature of many essential oils, combined with the fact that many essential oil components are naturally occurring in honey, makes such residue analysis difficult. Thus, the successful development of products employing essential oils can be extremely difficult unless the particular essential oil has been granted an exemption from existing regulations on maximum residue limits. In the EU, thymol, menthol and camphor have this status. The use of individual components of essential oils makes residue analysis much easier and limits the potential for producing off-flavour honey. Long-term studies have demonstrated that when used properly, residues of thymol in honey remain at low and safe levels (Tab.4).

## Conclusions

Based on the available studies, relying solely on one treatment per bee season with essential oils or essential oil components can not be recommended as an effective and reliable method to maintain mite populations below the economic injury level. The challenge for future research is to optimise the use of essential oils and essential oil components and to incorporate the resulting products along with other measures for limiting mite populations such as cutting out of drone brood, trapping combs, formation of nucleus colonies or the use of organic acids into an integrated pest management strategy for the control of *V. jacobsoni*. Adapting these strategies to local climatic conditions, to diverse apicultural management practices and to beekeeping operations of varying sizes pose additional and significant challenges. Finally, resistance to essential oils may eventually develop, as it has with synthetic pesticides. Consideration must be given to the development of resistance management plans to maximise the useful life span of effective acaricides and delivery systems once they are developed.

The results, reported in the present manuscript, are presented in detail in the review cited below.

## Literature

A complete literature list on this topic you will find in the following review:

Imdorf A., Bogdanov S., Ibáñez Ochoa R., Calderone N. W. (1999) Use of Essential Oils for the Control of *Varroa jacobsoni* in Honey Bee Colonies. Apidologie (30) 209-228

**Table 1 - Treatment of *V. jacobsoni* with pure thymol**

| Authors | year | thymol formulation | dosage | place of application | days of treatment | time of treatment | # of colonies | # of supers | # of apiaries | Type of hive | mean treatment efficacy % | mean treatment mitefall |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Marchetti et al. | 1984 | powder in bag | 4 x 15g | between combs | 16 | Oct./Nov. | 10 | 1 | 1 | Dadant | 66.0 | 3229 |
| Lodesani et al. | 1990 | powder | 3 x 4.5/6g | dusted over combs | 21 | Oct./Nov. | 38 | 1 | 2 | Dadant | 81.0 | 190 |
| Frilli et al. | 1991 | powder | 4 x 1g | on comb bars | 8 | Nov. | 7 | 1 | 1 | Dadant | 95.0 | |
| Chiesa | 1991 | powder | 5 x 0.5g/comb | on comb bars | 8 | Oct./Nov. | 21 | 1 | 3 | Dadant | 96.8 | 1917 |
| Liebig | 1995 | on compound | 2 x 15g | on comb bars | | Aug./Nov. | | 1 | | Zander | | |
| | | on compound | 2 x 30g | on comb bars | | Aug./Nov. | | 2 | | Zander | | |
| Higes et al. | 1996 | powder | 5 x 1g beeway | on comb bars | 19 | Feb. | 4 | 1 | 1 | Autocol. | 97.8 | 977 |
| Higes and Llorente | 1997 | powder | 4 x 8 g | petri on combs | 28 | Apr./May | 4 | | 1 | Langstr. | 97.6 | 1119 |
| Flores et al. | 1997 | powder | 2 x 10 g | petri on combs | | | | | | | 97.0 | |
| | | on compound | 2 x 10g | on comb bars | | | | | | | 95.0 | |
| Bollhalder | 1998 | on compound | 2 x 15g | on comb bars | 49 | Aug./Oct. | 22 | 1 | 4 | CH | 85.0-97.0 | |

**Table 2 - Treatment of V. jacobsoni with blends of thymol, eucalyptol, camphor and menthol** (N.C. = non commercial)

| **Authors** | **Year** | **product** | **number of com-pounds** | **place of appli-cation** | **days of treat-ment** | **time of treat-ment** | **number of colonies** | **number of supers** | **number of apiaries** | **type of hive** | **mean treatment efficacy %** | **mean treatment mitefall** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contessi and Donati | 1985 | Biovarroin | 2 x 1 | top | 35 | Nov./Dec | 2 | 1 | 1 | Dadant | 92.6 | 316 |
| Tonelli [88] | 1989 | Api Life VAR | 2 x 1 | top | | Nov./Dec | | | | | 93.8 | |
| Rickli et al. [80] | 1991 | Api Life VAR | 2 x 1 | top | 38 | Aug./Sep | 20 | 1 | 1 | CH | 96.4 | 986 |
| | | Api Life VAR | 2 x 1 | top | 79 | Aug./Oct | 20 | 1 | 1 | CH | 99.0 | 2453 |
| Mutinelli et al. [unpbl.] | 1991 | Api Life VAR | 2 x 1 | below | 40 | | 13 | 1 | 1 | Dadant | 89.0 | 593 |
| Van der Steen [91] | 1992 | Api Life VAR | 2 x1 | top | 42 | Sep./Oct | 5 | | 1 | | 74.0 | |
| | | N.C.+ | 2 x 1 | top | 42 | Sep./Oct | 5 | | 1 | | 92.0 | |
| | | N.C. - | 2 x 1 | top | 42 | Sep./Oct | 5 | | 1 | | 88.0 | |
| Moosbeckhofer [76] | 1993 | Api Life VAR | 2 x 1 | | 29 | Sep./Oct | 23 | 2 | 3 | Zander | 98.6 | 1400 |
| Mutinelli et al. [77] | 1993 | Api Life VAR | 2 x 1 | top | 49 | Aug./Oct | 27 | 1 | 4 | Dadant | 68.7 | 4925 |
| Liebig [59] | 1993 | Api Life VAR | 2 x 1 | top | | Sep./Dec | 14 | 1 | 4 | Zander | 97.4 | 1276 |
| | | Api Life VAR | 2 x 1 | top | | Sep./Dec | 26 | 2 | 4 | Zander | 63.9 | 1276 |
| Schulz [84] | 1993 | Api Life VAR | 2 x 1 | top | | Aug./Dec | 3 | 2 | 1 | Zander | 74.7 | |
| | | Api Life VAR | 2 x 2 | top | | Aug./Dec | 4 | 2 | 1 | Zander | 94.9 | |
| | | Api Life VAR | 2 x 3 | top | | Aug./Dec | 2 | 2 | 1 | Zander | 99.5 | |
| | | Thymix | 2 x 1 or | top | | Sep./Dec | 77 | 1 or 2 | 7 | Zander | 94.8 | 3492 |
| Imdorf et al. [50] | 1994 | Api Life VAR | 2 x 1 | top | 56 | Aug./Oct | 83 | 1 | 8 | CH | 97.7 | 602 |
| Imdorf et al. [46] | 1995 | Api Life VAR | 2 x 1 | top | 42-56 | Aug./Oct | 19 | 1 | 1 | Dadant | 91.7 | 1078 |
| Calderone and Spivak | 1995 | N.C. | 2 x 2 | top | 19 | Nov. | 8 | 2 | 2 | Langstro | 96.7 | |
| Gregorc and Jelenc | 1996 | Api Life VAR | 2 x 1 | top | 30 | Aug./Sep | 14 | 2 | 1 | Alberti. | 66.4 | |
| Loglio et al. [65] | 1997 | Api Life VAR | 3 x ½ | top | 21 | Jul./Aug. | 32 | 1 | 1 | Dadant | 72.6 | |
| Calderone [14] | 1999 | N.C. | 2 x 1 | top | 32 | Oct./Nov | 6 | 2 | 1 | Langstro | 67.0 | |

**Table 3 - Thymol residues in honey after different treatments**

| Type of thymol treatment | average mg/ kg | Min-Max mg/kg |
|---|---|---|
| **Thymol frame**<br>a whole year use in Switzerland, 1997 ( n = 22) | 0.33 | ≤ 0.02-0.83 |
| **Thymol frame**<br>a whole year use in Switzerland, 1998 (n=34) | 0.40 | 0.11-1.06 |
| **Thymol frame**<br>use outside the honey flow period in Switzerland 1998 (n=10) | 0.17 | ≤ 0.02-0.32 |
| **Thymol frame**<br>a whole year use in Germany, Wallner 1997 (n = 19) | 0.63 | 0.07-2.0 |
| **Api Life VAR**<br>8 weeks treatment in autumn, 1 to 5 use (n=28) | 0.16 | ≤ 0.02-0.48 |
| **Lime honey** (Guyot et al. 1998) | 0.08 | 0.02-0.16 |
| **Thymol concentration affecting honey taste** | 1.1-1.3 | |
| **Maximum residue limit for Switzerland** | 0.8 | |

# Varroa Control with Formic Acid

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For the control of varroa mites, formic acid has been applied by individual beekeepers for more than 20 years (2;25-28;35;47;52). Use of formic acid (FA) represents an essential part of an integrated management for the control of Varroosis.

## Mode of action

Formic acid has to evaporate within the beehive and it kills the mites by respiratory inhibition. To give full effect, high concentrations of FA in the air have to be present for several hours or even days. Short-term treatments need to reach higher concentrations for full efficacy, compared to long-term treatments. FA is the only chemical treatment which kills the mites both on the bees and within sealed brood cells (1;6;10;17).

## Application methods

### a. General

FA can be used successfully only when the ambient temperature is >12°C and sealed brood is present in the colonies. Efficacy and side effects depend on the dynamic of the evaporation of FA within the hive. Evaporation is influenced by

- ambient temperature/humidity
- colony size
- type of the beehive
- type of evaporation material (or wick)
- concentration of the FA applied

Because the large number of variables involved, standardisation of FA evaporation is difficult and results in large variations in efficacy among the application devices available on the market. In general, two types of application systems can be distinguished; short-term and long-term treatments. Depending on the method of treatment, a high concentration of formic acid for a few hours or a low concentration for several days is achieved in the air of the bee hive, with the respective system (14).

**b. Short-term treatments**

During the short-term treatment small amounts of formic acid evaporate relatively uncontrolled within 6 to 10 hours. At the beginning of the treatment the formic acid concentration in the hive air increases rapidly (Fig 1). Six hours later most of the acid has already evaporated. For proper use, the application method has to be adjusted to the ambient temperature and to the type of the hive used. When FA is applied from above 60% formic acid is used, whereas 85% may be necessary if the acid is applied from below (Tab 1). In Switzerland, the treatment in two blocks of two to three applications within a week in August after the honey harvest and in the end of September has proved to be efficient. The treatment efficacy obtained under these circumstances is approximately 90 to 95% (7;9;15;16;23;24;31;35;38;49;53).

The efficacy can be controlled by counting the natural mite fall starting two weeks after the last treatment by use of a bottom board with a metal screen covering the entire hive bottom. It is sufficient to count the mites once a week. If the natural mite fall is higher than one varroa mite per day, another treatment with oxalic, lactic acid or another acaricide is recommended (22). Six years'

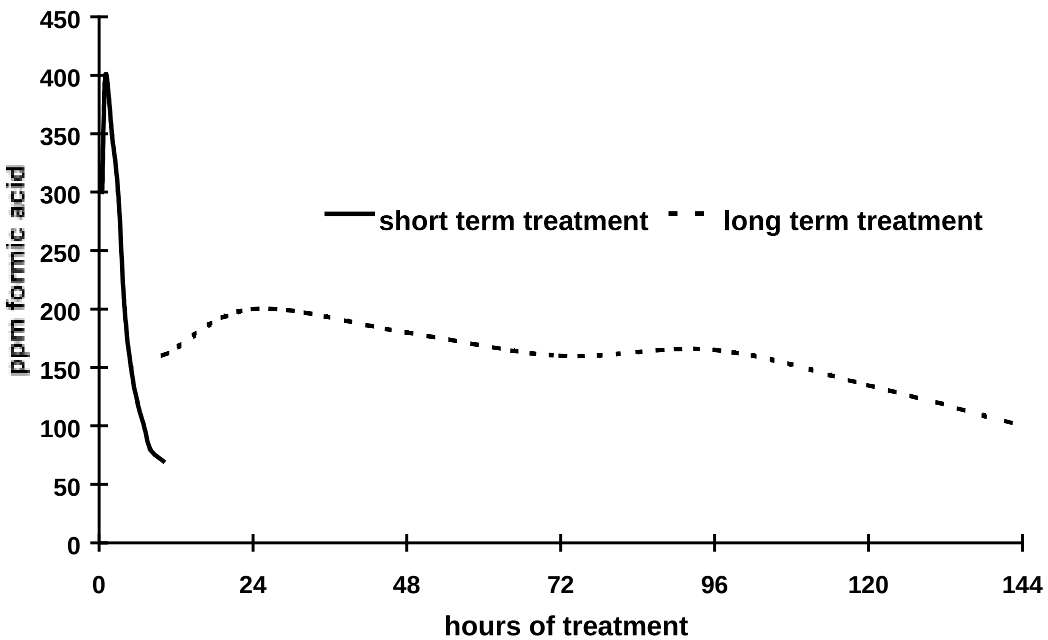


Fig 1. Concentration of formic acid in the air of the hive during the application of a short- and a long-term treatment (1ppm = 1.91 mg/m$^3$)

Experience with this method has shown, that follow-up treatments are necessary only after reinvasion in October (21). In order to avoid bee and queen losses, the indications below concerning temperature and application must be followed and the hive entrance has to be entirely open. Feeding at the time of the treatment can reduce side effects (but also efficacy).

Practical experience has shown, that short-term treatments alone often do not reduce the varroa population sufficiently to prevent a detrimental increase in the mite infestation level the next summer. To successfully rely on short-term FA treatments for mite control, it is necessary to reduce the increase of the varroa population also in the spring by removal of 2 - 3 drone brood combs or removal of sealed worker brood by forming nuclei.

Most short-term treatment methods do not require expensive devices or additional hive material and can be easily applied immediately after the last honey yield. Short-term treatments are an important module within an integrated concept to reduce mite infestation levels as early as possible during the season to prevent collapse of bee colonies and reinvasion of mites.

**c. Long-term treatments**

In general, there are two types of evaporation devices ("dispensers"):

Vacuum devices where FA is poured into a small container and evaporates through a wick. Examples are "Nassenheider" or "Medicine bottle" ("Tellerverdunster").

The FA is retained by an absorbent material and the evaporation is controlled by the surface only. Examples are "Krämer plate", "Apidea", “FAM-Liebefeld” and others.

In type 1 dispenser the acid concentration within the container keeps stable (no exchange with air humidity) and the amount of evaporated FA can be easily controlled.

Compared to several short-term treatments, the amount of work involved using long-term treatments is reduced considerably. Various types of evaporation devices for long-term treatments are available on the market. Also after long-term treatments an additional autumn/winter treatment with a systemic acaricide is recommended (depending on diagnosis of natural mite mortality) and/or removal of drone brood the following spring. By combining different control methods, it is not essential to achieve the highest possible efficacy using the FA treatment(s). A lower intensity of FA treatment can reduce the risk of queen losses considerably. Depending on the degree of infestation, one or two long-term treatments with formic acid are necessary. Under Swiss conditions, two long-term treatments are needed if natural mite fall is higher than 10 mites per day at the beginning of August. The first treatment over a one week period should be done immediately after the honey harvest. Depending on climatic region (ambient temperatures during the day should be > 15°C!) the second treatment of about two weeks should be done in mid-September. For colonies with a mite mortality of less than 10 mites per day, one FA treatment of two weeks in August/ September is sufficient (19).

Table 1 Application guidelines for short-term treatment with formic acid (FA)

| **ACTIVE SUBSTANCE** | **Formic acid – short-term treatment** |
|---|---|
| Application | Passive evaporation from absorbent material |
| Period of treatment | Begin: After honey yield<br>End: Depending on ambient temperature<br>Recommendation in Switzerland:<br>1st block of treatment ➔ beginning of August<br>2nd block of treatment ➔ end of September<br>(Duration of block of treatment app. 1 week) |
| Number of treatments | 2-3 treatments per block |
| Temperature during the day | 12-20 °C ➔ treatment during the day<br>20-25 °C ➔ treatment in the evening or morning<br>> 25 °C ➔ treatment early in the morning |
| Concentration | Treatment from the top ➔ 60 % FA<br>Treatment from the bottom ➔ 60% - 85 % FA(depending on ambient temperature) |
| Dosages<br>(depending of the size of the hive) | 1story (ml) / 2 story (ml)<br>From the top: 20-30 / 40-50<br>From the bottom: 20-30 / 40-60 |
| Absorbent material | Viscose sponge (slower evaporation)<br>Soft fiber cardboard (quick evaporation)<br>Soft fiber Pavatex plate (wood fiber) |
| Surface of evaporation | Approximately 15 x 20 cm |
| Control of treatment efficiency | Measuring of the natural mite mortality<br>Beginning ➔ 14 days after last treatment<br>Duration ➔ 2 weeks<br>More than 1 varroa/ per day ➔ additional treatment is recommended |
| Security measures by the application | Protective glasses, rubber gloves and water available |

## Side effects

### a. On bees

Queen losses were a severe problem at the beginning of FA treatments but have been significantly reduced to exceptional cases by correct use of modern application methods. Damages to open brood and hatching young bees, however, cannot be completely excluded. Damage rates depend on ambient temperature and the distance between the evaporation device and the brood. Under mid-European condition, a moderate loss of brood has no negative effect on colony overwintering [32].

**b. Residues**

Applied after the honey harvest in autumn, the natural content of formic acid in the honey of next spring may under certain conditions increase slightly (20;42;51). This increase has no negative effect on the honey quality (5).

## Efficacy and recent experiences under field conditions

The efficacy of one FA treatment will range from about 60% to 80%. For two applications, the efficacy can increase to 90-95%. The efficacy has been extensively tested for the Krämer Plate (8;29;30;39;50), the Nassenheider (3;4;7;36;43-45), the FAM-Liebefeld dispenser (12;13) and in recent times the Tellerverdunster (32-34;48) . In comparative tests in three apiaries (11) the efficacy of 5 dispensers were between 92 and 98% (two treatments). This efficacy can be regarded as sufficient to obtain control of the mite population when used in an integrated system (Tab 2). More dispensers such as the Universalverdunster (37) and the gel application (40) where an efficacy of more than 90% can be expected, has recently been developed.

Although the average effect of FA treatments may be acceptable, there are often colonies or apiaries with insufficient treatment results because of variations in efficacy. Such colonies or apiaries represent a risk for other colonies because of reinfestation.

An important point is that the application of FA has to be included intensively in the beekeeper extension work and that details of the method has to be adapted to the local conditions (climate, bee hive). It will not be possible to develop a general application guideline for all of Europe as we are confronted with a wide range of climatic conditions, hive types and different bee management system. Finally it seems that under Mediterranean conditions FA application is more difficult, probably because of the high and often varying temperature.

*Tab 2 Efficacy of formic acid against* Varroa jacobsoni *after the application of 2 long-term treatments with 5 different dispensers.*

| Dispenser | Colony number | Efficacy (%)<br>1st FA treatment | 2nd FA treatment | Total FA |
|---|---|---|---|---|
| **Apidea** | 14 | 59 | 89 | 96 |
| Burmeister | 14 | 42 | 87 | 92 |
| FAM-Liebefeld | 13 | 74 | 91 | 98 |
| Krämer Plate | 13 | 37 | 92 | 95 |
| Wyna-Deluxe | 10 | 75 | 85 | 96 |

## Conclusion

| Advantages | Disadvantages |
|---|---|
| Useful in colonies with brood | Variation of efficacy (as exclusive treatment method mostly not sufficient) |
| No negative effects on honey quality | Minor brood damages have to be accepted |
| No resistance of the mite reported so far | Several treatments necessary |
| Important module for integrated concepts | To many methods on the market ("confusion") |
| 15 years experience in Europe available | Requirement of beekeeper extension with adaptation to local conditions |
| Also toxic for tracheal mites (18;41;46;54) | |

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# Biotechnical Control of Varroa Mites.

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## Introduction

Different methods for biotechnical control of varroa mites in honey bee colonies have been tested by institutes in Sweden, Germany and the Netherlands and the results have bee discussed during the meetings of CA3686.

The possibilities to control the mites without use of chemical treatments through biotechnical means only, are very limited. Biological control agents have not yet been found and the only physical method developed so far (heat treatment) requires sophisticated and expensive equipment. The most successful biotechnical methods are all methods that trap the mites in worker or drone brood with subsequent removal of this brood from the colonies.

The immediate effect on the varroa population in infested colonies when trapping combs are used is fairly well known. However, to enable the use of trapping methods in integrated control schemes, it is also important to know more about the development of the mite population over a prolonged period of time when biotechnical methods are combined with other control measures. At the moment long time studies are underway that combine trapping methods and treatments with organic acids.

## Methods

The terminology “trapping methods” is somewhat confusing. Here the most commonly used English terms and their German equivalents are used.

There has been an evolution of methods. In the sixties it became clear in Russia that drone brood was more attractive to varroa mites than worker brood, and since then drone brood was used in the spring to remove mites from colonies. Grobov (1977) reported that 20 to 30 percent of the mites could be removed from the colonies by means of a building frame, which gave the bees the possibility of building combs with drone cells only. Later this method was also used in the rest of Europe and it is still in use today to slow down population build-up of varroa in spring (drone brood removal, Drohnenbrut Entnahme).

Maul (1983) in Germany developed a method to concentrate worker brood on a limited number of frames, which subsequently could be removed (trapping comb method, Bannwabenverfahren). Worker brood trapping combs were produced between two queen excluders. The queen is kept in captivity on one or more combs by means of these queen excluders for seven to nine days. Then she is moved to the next empty comb(s) etcetera. The brood is removed as soon as it is capped. This method controlled mite populations in Sweden when combined with drone brood removal (Fries and Hansen, 1989)

Büchler (1997) later modified trapping comb method used by Maul (1983) by using drone comb instead of worker comb.

Boot and Calis studied the invasion behaviour of varroa in worker and drone brood (Boot, 1995). Based on their calculations the prediction was that the effectiveness of trapping varroa mites in drone brood could be improved dramatically if no worker brood was present at the same time. A limited number of drone cells should be enough to capture over 95% of the mites, provided the cells were capped at the right time. If colonies are managed so that there is no sealed brood present and no worker brood available suitable for invasion, and drone brood ready to be sealed is added to such colonies, the trapping efficacy is very high. If drone brood removal is integrated in the beekeeping method, making use of broodless period created by the beekeeping practice, we call this option for mite control the drone brood method (Drohnenbrut Methode).

Several drone brood methods have been developed to fit into the local swarm prevention methods widely used in The Netherlands. If the method is combined with measures necessary for swarm prevention, the amount of extra work is limited.

## Effectiveness of the different methods

Several authors reported 20 to 30% effectiveness of drone brood removal in the spring (Eijnde 1983; Grobov 1977; Rosenkrantz and Engels 1985; Schulz et al. 1983). With the summer ahead, this is not enough to refrain from other control measures until the following spring. However, population build-up is slowed down considerably when drone brood is removed and because the method is simple and not very laborious, it can be combined with other control measures.

With the trapping comb method over 96% efficacy can be reached if four worker combs are used subsequently (Maul et al. 1990). With three worker combs the effect was 93.5% in German studies (Klepsch et al. 1983). With three combs of drone brood the effectiveness is better than with worker brood (Büchler 1997a, 1997b). The trapping comb method requires specially made queen excluders and is labour intensive (the queen has to be confined).

Using the drone brood method, between 93% and 95% of the mites could be removed from the colonies in Dutch studies (Calis et al. 1997).

## Long-term effect

At Ambrosiushoeve in the Netherlands we started a long-term experiment with a group of 20 colonies in the spring of 1998. The colonies were treated with the drone brood method as described by Calis (1997). All colonies were provided with a gauze "Varroa bottom" and a drawer to facilitate the collection of dead mites. Dead mites were collected weekly. The total number of colonies varied during the season, because of splitting colonies in the spring and uniting them again in the autumn. Of the total group of colonies, the average number of dead mites per colony per week is given in figure 1.

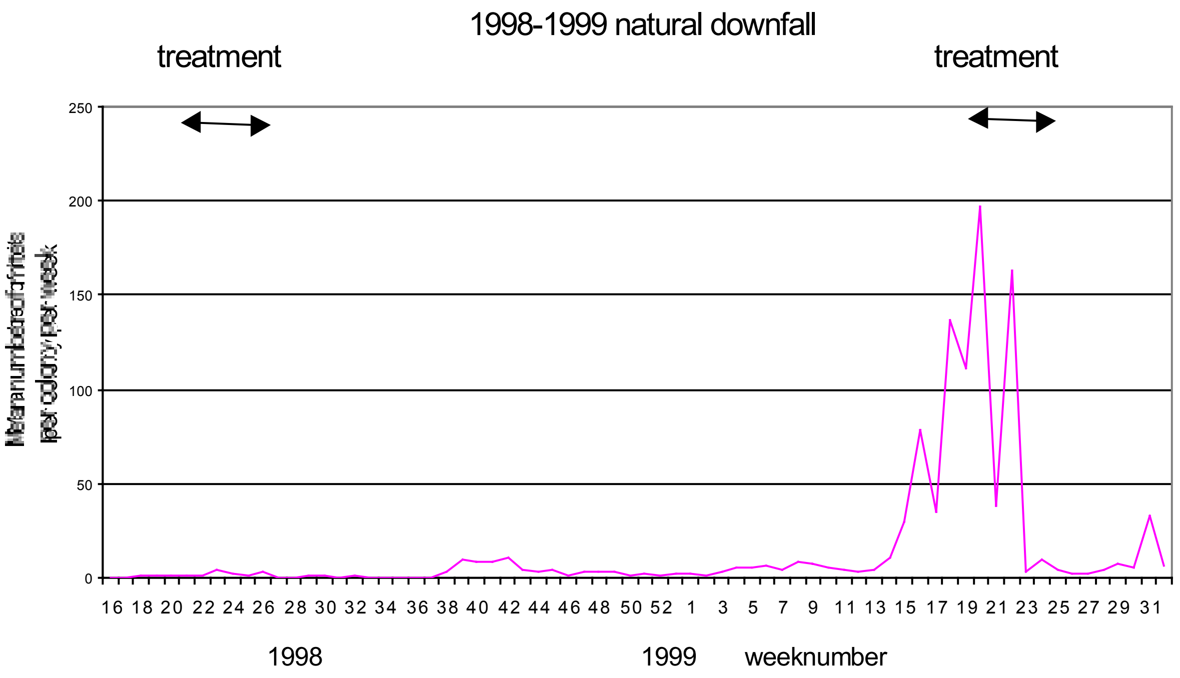


Figure 1. Average natural mite mortality in colonies treated with the drone brood method in 1998 and 1999.

Starting with a low number of mites in spring 1998, the average numbers remained low after application of the drone brood method. No other measures against varroa were taken during the first year. In the spring 1999 a rapid increase of natural mite mortality was observed. After application of the drone brood method for the second time, the natural mite mortality dropped again. Compared to the same weeks in 1998, we see a slight increase. This means that the drone brood method alone is not sufficient to keep the infestation at a low level over several years and that supplementary measures are necessary. In an integrated schedule a treatment using formic acid in August could be used or treatment with lactic acid in brood less colonies during late fall/winter. Combining methods have the potential to reduce infestation levels to a level low enough to avoid damage to the colonies until the next application of the drone brood method in the spring 2000.

Büchler compared the trapping comb method (with drone brood) as described above, with the drone brood method. He started with two equally strong groups of colonies in June 1998. In his drone brood method all brood but 1 comb with open drone cells was taken out of the colonies, together with a part of the younger bees. Only the results of the remaining colonies were taken into account. The absolute numbers of mites removed from the colonies was low, but the natural mite mortality stayed low during the rest of the year, so no further treatments were needed. The population check according to the Liebefeld method showed that the colonies in the drone brood group were weaker in the autumn, but stronger in the next spring. The treatments were repeated in the spring of 1999 and the colonies are still in good condition.

## Conclusion and recommendations

All trapping methods require several actions per colony and, thus, are labour intensive. Trapping comb methods have some disadvantages compared to drone brood methods, because of the use of queen excluders and the need to find and handle the queen several times.

Drone brood methods are highly effective and can be recommended to beekeepers with a limited number of hives, especially if the method can be combined with swam prevention techniques. None of the described trapping methods can reduce the number of mites sufficiently on its own. Additional methods (like a formic or a lactic acid treatment) are necessary to avoid damage until the next opportunity to apply the drone brood method.

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# Residues of Varroacides in Honey, Bees Wax and Propolis

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## Summary

The use of varroacides in bee colonies leaves residues in various bee products. Among the variety of available varroacides, three ingredients are commonly detectable in honey and beeswax: bromopropylate (Folbex VA Neu/ Acariol), coumaphos (Perizin/Asuntol), and fluvalinate (Apistan/Klartan/Mavrik). These chemicals are fat-soluble and non-volatile, and thus they accumulate in ppm levels as residues in beeswax with years of treatment. Through the process of diffusion, these ingredients migrate from the wax comb into the stored honey.

In recent analysis of German honey samples, the most frequently found varroacide is coumaphos (28%). Bromopropylate is detectable but with decreasing frequency (11%). Because of its high binding strength in beeswax, detection of fluvalinate is relatively rare in honey (1%). All residues were found with low ppb levels. Other ingredients with similar chemical behavior, presently play an unimportant role as residues in honey, beeswax, and propolis due to the very low amount of ingredients used (acrinathrine, flumethrine) or instability (amitraz).

## Introduction

In many parts of the world, the threat of infestation by the parasitic mite, *Varroa jacobsoni* Oud., forces beekeepers to treat their colonies with acaricides. Currently, there are many preparations and procedures available to treat the mite. Depending on their national registration, the substances that can be used as treatments vary. Since it is the purpose of these regulations to protect the users and consumers, an international standard among national regulations is needed.

Beekeepers and various industries benefit from the healthy and pure image that bee products present to the public. To protect this image, it is important to minimize or eliminate the residues of varroacides left behind in products such as honey, in which purity and quality is expected.

## The chemical behavior of varroacides

The active ingredients of varroacides can be divided by into two main groups: the water-soluble (hydrophilic) and the fat-soluble (lipophilic) ingredients.

Water-soluble, active ingredients such as formic acid, oxalic acid and cymiazole endanger the quality of honey since these substances are easily diluted in honey. Use of such substances during the nectar flow always results in considerable amounts of residues. Also, organic acids may introduce a false taste to the honey. However, volatile residues, such as formic acid, decrease in stored food and extracted honey over time (Capolongo et al., 1996; Imdorf & Charrière, 1998; Stoya et al., 1986) Water-soluble ingredients have no negative long-term effect on beeswax quality since they are not stored in beeswax.

The situation is different in the case of fat-soluble substances. First of all, these ingredients are stable and increase in the wax comb. Fat-soluble ingredients are distributed throughout the colony by the bees' legs and bodies. All inner surfaces of the hive, which are walked on by bees such as the frames, bottoms, and covers, are coated with a very thin layer of wax. Lipophilic substances are stored there and may pass in measurable amounts into other bee products like honey, virgin wax and propolis. Laboratory samples given the same conditions as in the bee colony have clearly demonstrated this migration (Wallner, 1992). The higher the concentration in the wax, the more residues could be detected in honey in contact with the wax. Another negative effect is the contamination of wax particles in the honey (Wallner 1995). Contaminated wax is a significant source of residues in honey because a natural degradation of varroacides in beeswax does not occur. Rather, there is an accumulation of lipophilic varroacides in beeswax due to repeated applications. The effect from wax contamination on the mites seems to be negligible unless extreme values are present (Fries et al., 1998). Beeswax has a large capacity to hold contaminants and the recycling of old wax combs into foundations does not change the content of the active ingredient to any significant degree (Bogdanov et al., 1997). Also, the technical capabilities for cleaning of wax are limited (Vesley et al, 1994; Wallner, 1995). Only the complete destruction of beeswax by, for example, burning the wax as a candle will destroy the stored ingredients (Wallner, 1998). In sum, fat-soluble ingredients, especially when they are stable and non-volatile, represent a great risk in apiculture of long-term residue accumulation.

Within the group of fat-soluble active ingredients, there are varroacides whose concentration can decrease in beeswax. This decrease is because they contain semi-volatile ingredients such as essential oils (e.g., thymol, wintergreen oil), and other substances, which can decay into metabolites (e.g., amitraz). During the application of the treatment, only some of the semi-volatile ingredients attach to the wax, while a majority evaporates because of the temperature in the hive (Imdorf et al., 1995). As a result, the amount actually left in the wax is effectively reduced. Although, an accumulation of residues does not occur during years of application, traces may remain detectable in honeycomb (Bogdanov et al., 1998) The amount of semi-volatile ingredients can also be effectively reduced during the recycling of old wax into foundations, if the wax is not only liquefied but is also steamed.

## Residues in Honey

With few exceptions, honey analysis in different laboratories have demonstrated that residues of the stable, lipophilic substances can be found. Up to now, the following varroacides have been detected with ppb levels in honey: bromopropylate, coumaphos, fluvalinate, malathion, diazinon, chlordimeform and cymiazole (Wallner, 1999).

Since 1988, long-term studies have been carried out at the University of Stuttgart-Hohenheim, in which up to 1,000 honey samples are analyzed per year within the scope of general quality control. In German honey, residues of three synthetic varroacides are detectable. They all belong to the group of non-volatile, fat-soluble substances.

Bromopropylate (Folbex VA Neu, Acarol).

Although this ingredient has not been used in Germany for 8 years, bromopropylate residues can be found in amounts of 2-10 ppb in approximately 11% of German honey. In 1996, the percent of positive samples was 17.4%, and in 1995 it was 20.2%. Without exception, these residues come from accumulation of the active ingredient in wax combs, on frames and hive walls, and from foundation made from contaminated wax. In the past, residues of bromopropylate were detected in honey with levels above 100 ppb.

Coumaphos (Perizin/Asuntol)

Today, Perizin is the most frequently used trickling solution in German apiaries. It is mainly used during the winter in colonies without brood, but sometimes it is also used in late summer in multiple treatments in colonies with brood. Coumaphos represents the most frequently detectable varroacide in honey. In 1997, approximately 28% of examined honey was contaminated with levels between 2-15 ppb. Similar residue levels were found in 1995 and 1996 in 24.5% and 31% of the analyzed samples, respectively.

Fluvalinate (Klartan/Mavrik/Apistan)

Fluvalinate belongs to the group of synthetic pyrethroids. It is used for control of *V. jacobsoni* world-wide. Impregnated carriers are inserted into the colony with amounts of fluvalinate in grams. In drip solutions, the needed amount of ingredient is extremely low in comparison to other systemic varroacides (coumaphos, cymiazole). Residues in German honey are found only rarely when mistakes occur in the application and/or in preparing the trickling solution, and if residues in the wax are at a high level. In these instances, the substance migrates by diffusion from the comb into the honey. Fluvalinate can be found in 1% of honey produced in Germany with residue levels between 2-7 ppb. Higher amounts reaching 40 ppb were found in honey from Eastern Europe, and are reported from other countries, as well (Kubik et al., 1995). Recently, fluvalinate resistant mites have appeared in several countries, which indicates that the continued use of the whole group of synthetic pyrethroids is questionable (Milani, 1995). The remaining synthetic varroacides play a subordinate role in residues in German honey.

## Residues in Wax

Most of the fat soluble substances, with the exception of amitraz, are widely used as varroacides and can be found with ppm levels in beeswax. Up to now, the following varroacides have been detected in wax: bromopropylate, coumaphos, fluvalinate, flumethrine and tetradifon (Wallner, 1999).

Because of the very small amount of substance used, levels of acrinathrine and flumethrine in beeswax from apiaries can be expected to be below the detection limit of most laboratories. Since 1993, 300-400 beeswax samples have been analyzed per year at the University of Hohenheim. Most of these samples were received directly from beekeepers.

## The current situation

The residue situation is not homogeneous, since the concepts for mite treatments vary greatly among beekeepers. The active ingredients listed above are detectable in beeswax and as residues in honey. In wax, the measured values of bromopropylate, coumaphos and fluvalinate are in the ppm area (detection limits 0.5 mg/kg). More than 90% of the examined local samples are contaminated. Often several of these varroacides can be found in the same sample. However, there is an increasing number of beekeepers who have stopped using fat-soluble ingredients and use organic acids exclusively. No residues can be found in the wax from these apiaries, provided that residue free foundation is used.

As expected, German wax is contaminated with high percentages of bromopropylate (54.9%) and coumaphos (61.0 %). A large number of samples contain amounts between 1-5 ppm. On the other hand, more than half of the international samples (55.0%) are contaminated with fluvalinate. This active substance can also be found with increasing frequency in German wax. In 1996, the percentage of positive samples was 23.6% and in 1995 only 13.2%. Similar frequencies with slightly higher levels were shown in other countries

(Bogdanov et al., 1990; De Greef et al., 1994; Pechhacker & Wallner, 1991). As a fat-soluble, non-volatile substance, fluvalinate plays the chief role as a residue creating substance in beeswax. The same frequencies are also found in wax foundation on the national and foreign market, as beeswax is an internationally traded product.

In general, residues of varroacides in beeswax are not regulated. With the exception of the USA (fluvalinate 6 ppm), no official limits exists. Since great amounts of beeswax are processed for pharmaceutical purposes or for the food and cosmetics industry, pesticide residues are problematic. Several companies have created their own internal acceptable maximum limits of wax contaminants. Because of the easy migration of substances into honey, wax used for honey production should have the least amount of contamination. To ensure that there is no measurable effect, residues in the wax should be lower than 1 mg/kg (Wallner, 1995).

## Residues in Propolis

Little data exists on the influence of varroacides on the quality of propolis. Propolis that is mainly used in apitherapy should be free of measurable pesticides. The available data show that propolis has a high affinity to fat-soluble varroacides and is susceptible to contamination. For example, bromopropylate was detected with levels >11 ppm after fumigation (Stehr et al., 1996) and fluvalinate had levels >8 ppm (Kubik et al., 1995), in single samples of more than 50 ppm (Wallner, 1995) after using Apistan strips. Alcoholic extractions of contaminated rough material also contained residues, independent of the alcohol concentration (Wallner, 1999).

## Conclusion

The treatment of colonies against *V. jacobsoni* influences the quality of bee products in many countries of the world. Depending on the chosen varroacide, a varying level of residues can be found in honey, beeswax, and propolis. Discussions on the toxicology of these residues are endless, as the current official limits do not contribute to an objective evaluation of the topic. In the future management of honeybees, it is important to consider that as soon as there is a choice between contaminated and residue free products, consumers will choose the latter.

The use of synthetic, lipophilic varroacides in colonies should be minimized, and the use of organic acids or essential oils increased. It is also necessary to change habits of recycling wax into foundation. Old combs that are contaminated should not be used for the production of foundation. Instead, foundation should be made from virgin wax and wax cappings. With an increased production of virgin wax in colonies, an efficient acaracide application system, and a system to separate contaminated combs from the wax recycling process, residue levels in bee products can be maintained below the detectable limits and far below the maximum residue levels.

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# Rules Governing the Use of Products for Varroa Control

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## Summary

European Union regulatory needs for veterinary medicinal products have now been fulfilled by a new authorisation system. This system provides access to a Europe-wide market for innovative products and facilitates access to all Member States for other products. This includes medicines used for the control of *Varroa jacobsoni* infestation of honeybees.

Harmonization of the requirements for veterinary medicines began in 1981 with the adoption of the Directives 81/851/EEC (covering manufacturing) and 81/852/EEC (covering marketing), based on quality, safety and efficacy evaluation of products. Additional measures harmonised the procedures and criteria used to evaluate veterinary medicines.

However, granting of authorisations still remained national. Consequently, although applications were evaluated on the basis of these harmonised procedures, there were differences in the decisions reached by Member States on individual products. So the Commission made proposals in 1990 for a new marketing authorisation system which was adopted by the Council of Ministers in 1993, and came into force on 1 January, 1995. One result was the creation of the European Agency for the Evaluation of Medicinal Products (EMEA) in London.

Council Regulation (EEC) 2377/90 laid down a Community procedure for the establishment of Maximum Residue Limits (MRLs) of veterinary medicines in foodstuffs of animal origin. In practice, it bans all veterinary drug residues in food, including honey, except those that have been approved.

Since 1995 two new EU registration procedures for human and veterinary medicines have been introduced: the centralised and the decentralised (mutual recognition) procedures. In accordance with Directive 81/851/EEC, authorisation is also required for manufacture of veterinary medicines. Close relations are maintained with the licensing authorities of the European Economic Area, where integration is developing via the implementation of common directives and guidelines for human and veterinary medicines. Also, the Pharmacopoeia Discussion Group (PDG) was co-founded in 1990 by the European Pharmacopoeia, the Japanese Pharmacopoeia and the United States Pharmacopoeia. This group is working for world-wide harmonisation.

## Introduction

The human and veterinary pharmaceutical legislation of the European Community has evolved over a 30-year period. Harmonisation of the requirements for veterinary medicines began in 1981 with the adoption of the Directives 81/851/EEC (covering manufacturing) and 81/852/EEC (covering marketing), based on quality, safety and efficacy evaluation of products. Additional measures harmonised the procedures and criteria used to evaluate veterinary medicines, such as framework requirements and interpretative guidelines for testing, guidelines for Good Manufacturing Practice (GMP), and a community procedure for the evaluation of high-technology products (Pastoret and Falise, 1999).

However, granting of authorisations remained national. Consequently, although applications were evaluated by these harmonised procedures, there were still differences in the decisions reached by Member states on individual products. So the Commission made proposals in 1990 for a new marketing authorisation system which was adopted by the Council of Ministers in 1993, and came into force on 1 January, 1995. One result was the creation of the European Agency for the Evaluation of Medicinal Products (EMEA) in London.

## European Agency for the Evaluation of Medicinal Products (EMEA)

In 1995 a new European Union system for the authorisation of medicines came into force. After ten years of co-operation, and four years of negotiations, between member state registration authorities, the EU Council adopted three directives and one regulation in June 1993, which together form the legal basis of the new system (Brunko, 1997).

The EMEA was established by Council Regulation (EEC) 2309/93 of 22 July, 1993 (OJ L214, 24.8.1993) and set up in London. Its mission statement is to contribute to the protection of public and animal health by:

- mobilising scientific resources throughout the EU to provide high quality evaluation of medicines, to advise on research and development programmes and to provide useful and clear information to users and health professionals;
- developing efficient and transparent procedures to allow timely access by users to innovative medicines through a single European marketing authorisation;
- controlling the safety of medicines for humans and animals, in particular through a pharmacovigilance network, and the establishment of safe limits for residues in food-producing animals.

The EMEA is comprised of a management board, two scientific committees (One in charge of human medicines and one in charge of veterinary medicines) plus the necessary administrative staff.

The CVMP is responsible for marketing authorisation evaluations for biotechnology derived products, for productivity enhancers, new chemical entities intended for use in food-producing animals, and other innovative new products. In addition it makes recommendations on MRLs (Maximum Residue Limits) for substance used in food-producing animals according to Council Regulation (EEC) 2377/90 of June 26, 1990 (OJ L224/1-8, 18.8.1990).

The CVMP has appointed a number of working parties chaired by some of its members: safety of residues; efficacy; immunological veterinary medicines; pharmacovigilance; joint CPMP/CVMP quality. Considerable effort has been put into developing guidance notes on a variety of topics.

Transparency is a major objective of the agency. For example, on its web page (http://www.eudra.org/), the EMEA makes available the following:

∑ European Public Assessment Reports (EPARs) for all centrally approved products, and

∑ Summary Reports for Maximum Residue Limits for Veterinary Medicines for food animals

The reports are to reassure the public about the work of the agency secretariat and its scientific committees (Jones, 1999). To support its activities, the CVMP relies on a pool of 400 experts out of over 2000 now accredited, put at the disposal of the agency by the EU Member states. The guidelines for the testing of veterinary medicines are in Volume VII of the rules governing medicinal products in the EU, published by the European Commission in 1994.

## Maximum Residue Limits (MRLs)

Council Regulation (EEC) 2377/90 laid down a Community procedure for the establishment of MRLs of veterinary medicines in foodstuffs of animal origin. In practice it bans all veterinary drug residues foods, including honey, unless they have been approved (Martin, 1999).

However, a distinction must be made between a basic veterinary medicine and the final preparation in which it is sold. Medicines are potent by design and may be safe for use only if presented to the diseased animal in a particular form. That preparation has to receive a marketing authorisation (previously called a product license) under EC medicines legislation. An application to the authorities for such an authorisation must include evidence of efficacy and safety, supported by results of toxicological, metabolic and residue studies.

Each member state had its own authorisation system before the creation of the EMEA but without common MRLs. Nevertheless, a product with national approval that was on the market on 1 January 1992 will be allowed to remain on the market until the end of 1999. This is provided that its pharmacologically active ingredient is on the so-called list of 'defended substances' (communication of the EMEA pursuant to Article 1 of the Council Regulation (EC) 434/97; OJ C165/03, vol. 40, 31.05.1997). By 31 December 1999 all pharmacological active substances must be included in Annex I, II or III of Council Regulation (EEC) 2377/90 or the product must be removed from the market (Table 1 and 2).

### *Available European Licensing Procedures*

Since 1995 two new registration procedures for human and veterinary medicines have become available through the EU: the centralised and the decentralised procedures.

The centralised procedure is compulsory for medicines derived from biotechnology (Part A), and available on request for other innovative new products (Part B). Applications are submitted directly to the EMEA. At the conclusion of a scientific evaluation (taking up to 210 days), the opinion of the scientific committee is given to the Commission to be transformed in into a single market authorisation (taking a further 90 days) which applies to the whole of the EU.

Applications may be submitted under the rules of Part A or Part B of the annex to the regulation. Article 3 of Regulation 2309/93 states that no medicinal product referred to in Part A of the regulation may be placed on the market without being submitted to the centralised procedure. On the other hand, the person responsible for placing a medicine on the market according to Part B may ask for the centralised procedure.

The decentralised procedure applies to the majority of conventional medicines and is based on the principle of mutual recognition of national authorisations. It allows the extension of a marketing authorisation granted by one member state to any other member states identified by the applicant. Should the original national authorisation not be recognised by another member state the EMEA acts as the arbitrator. In this case, the European Commission adopts the final decision with the assistance of the regulatory committee or, in the event of serious disagreement between the member states, with the assistance of the Council of Europe. National procedures remained available to applicants during the transitional period (up to 1 January 1998) after which they may be used exclusively for nationally marketed medicinal products, the decentralised procedure being mandatory.

Member states recently created a Mutual Recognition Facilitation Group (MRFG) to facilitate admission of medicines under the decentralised procedure. Meetings are held monthly at the EMEA chaired by United Kingdom.

### *Manufacturing Authorisation*

In accordance with Directive 81/851/EEC, authorisation is also required for manufacture of veterinary medicines. This directive provides for regular inspections and insists that manufacture must be supervised by a "qualified person", who certifies that each batch meets with the approved specifications for the product. These requirements are implemented by Directive 91/412/EEC, which provides the principles and guidelines of Good Manufacturing Practice (GMP). The Commission has published a detailed guide on GMP, developed by a group of pharmaceutical inspectors from member states.

### *European Pharmacopoeia*

Profound changes in the organisation of the European people and the regulation of medicinal products have characterised the last decades (Artiges, 1997). Thirty years ago each country had its own regulations and between them the European countries had two thirds of the world's pharmacopoeias with all possible variations. The European Pharmacopoeia Convention has now been signed by 24 parties: 23 countries plus, just recently, the Commission of the European Communities. Moreover 10 European and non-European countries and the World Health Organisation (WHO) have observer status. Close relations are maintained with the licensing authorities of the European Economic Area, where integration is developing via the implementation of common directives and guidelines for human and veterinary medicines. Also, the Pharmacopoeia Discussion Group (PDG) was co-founded in 1990 by the European Pharmacopoeia, the Japanese Pharmacopoeia and the United States Pharmacopoeia. This group is working hard for world-wide harmonisation.

During the 1960s Europe agreed to pool technical and scientific expertise, mainly within the framework of the two major international organisations, the EU and the Council of Europe. This led to the formation of a coherent body of regulations covering marketing and quality control of medicines for human and veterinary use, manufactured locally or imported. Its main components are marketing authorisation, granted case by case for medicines manufactured industrially, and the Pharmacopoeia, a tool for standardisation. The European Economic Community elaborated the regulations surrounding marketing authorisation after extensive public consultations with professional pharmaceutical associations as well as European Free Trade Association (EFTA) countries and the Nordic countries. The European

Pharmacopoeia was developed under the *aegis* of the Council of Europe by means of a specific international convention, which from the start allowed a larger number of European countries to participate.

The convention on the Elaboration of a European Pharmacopoeia is based on a dual commitment by its signatory states:

∑ a commitment to elaborate a common pharmacopoeia, by contributing financially to its budget and by sending experts;

∑ a commitment to make official on their territories, the specifications of the European Pharmacopoeia replacing, where applicable, the existing national requirements.

This commitment is now official, and has been integrated into the regulations for the registration of medicines manufactured industrially since the adoption in 1975 of the first directive (75/318/EEC) on the standards and protocols for analytical, pharmacotoxicological, and clinical studies on medicines for human use. This principle has also been applied to veterinary drugs by Directive 81/852/EEC and also to immunological products (Directive 89/342/EEC), to veterinary vaccines (Directive 90/677/EEC), and to homeopathic medicines for human use (Directive 92/73/EEC) and for veterinary use (Directive 92/74/EEC).

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Table 1. Authorised substances and their limits

| **Veterinary drug** | *MRL* |
|---|---|
| Fluvalinate (Apistan) | **NCN**[1] |
| Flumethrin (Bayvarol) | NCN |
| Formic acid | NCN |
| Lactic acid | **NCN** |
| Menthol | **NCN** |
| Thymol | NCN |
| Eucalyptol | NCN |
| Camphor | NCN |
| Mixed oils (ApilifeVAR)[2] | NCN |
| Cymiasole (Apitol) | 1000 mg/kg (ppb)[3] |
| Amitraz (Apivar) | 200 mg/kg (ppb)[3] |
| Coumaphos (Perisin) | 100 mg/kg (ppb)[4] |

[1] NCN = none considered necessary
[2] A proprietary mixture of the four listed essential oils
[3] Provisional MRL expires on 01.07.1999
[4] Provisional MRL expires on 01.07.2001 (Opinion of the CVMP, January 13, 1999)

Table 2. Products used in the European countries for the control of *Varroa jacobsoni* infestation

| Country | Registration as pesticide | Registration as veterinary medicine* | No requirements (free use) | Not registered, accepted/tolerated/ temporary authorisation |
|---|---|---|---|---|
| **Austria** | Apistan, Bayvarol | | | FA, LA, OA |
| **Belgium** | Apistan | Apivar | | OA, FA, LA |
| **Switzerland** | | Apistan, Bayvarol, Perizin, Apitol, Illertisser mite plate Api Life VAR, Thymovar | formic, lactic and oxalic acid, thymol | OA, FA, LA, thymol |
| **Germany** | | Perizin, Apistan, Apitol, Bayvarol, FA (Nassenheiderverdünster), Illertisser mite plate | | LA, thymol, |
| **Denmark** | None | none | | Annex II EC Regulation 2377/90 |
| **France** | | Apistan, Apivar | | Annex II EC Regulation 2377/90 |
| **Finland** | Apistani | | | |
| **United Kingdom** | | Bayvarol, Apistan | | **ii** |
| **Republic of Ireland** | | Bayvarol | | |
| **Greece** | | Apistan, Perizin, Apitol, Folbex | | |
| **The Netherlands** | | Apistan, Apitol | | OA, FA, LA |
| **Italy** | Perizin, Apitol, Folbex VA, Api Life VAR, Apistan, Bayvaroliii | Apivar | | FA, LA, OA, thymol |
| **Norway** | none | | FA, LA, OA | |
| **Portugal** | Apistan | Apivar | | |
| **Sweden** | Apistan | | | Essential oils, FA, LA, OA |
| **Slovenia** | Apistan, Api Life VAR | | | |
| **Spain** | Apistan | Perizin, Apivar | | OA, FA, LA, thymol, rotenone |

* Directives 81/851/EEC, 90/676/EEC, 81/852/EEC, 87/20/EEC, 94/40/EEC, 93/41/EEC;

**NOTES:** 1 Decision made by the pesticide regulation board of Ministry of Agriculture on 29.04.1998: “Products containing formic acid, oxalic acid or essential oils have to be registered as a pesticide, if used in the control of varroosis or acarioosis” these products are not allowed to be sold or used, if they are not registered as a pesticide.

1 Any “traditional” product provided it does not have toxic residues. Defined as “Non-Medicinal Curative Substances”.

1 Their re-registration as veterinary medicine is in progress.

# Ecological Varroa Control

notes on control strategies for North Europe

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Varroa control must become an integrated part of the management. To get sufficient control different methods are combined and used on more than one occasion during the season.

Concepts are mainly based on organic acids and biotechnical methods.

## Drone brood removal

By removing sealed drone brood in spring/early summer the infestation level can be reduced during the active season. There is a number of different types of frames and systems that can be used, and drone brood removal can be prepared for either in spring or in the previous fall by installing drawn drone comb in the winter cluster. In addition to reducing the infestation level drone brood removal also reduces swarming. It is important to remove combs before hatching of drones, otherwise mite population build up increases rapidly.

## Formic acid

Formic acid kills mites both in sealed brood and on adult bees. A short-term treatment applied directly after honey harvest (possibly during feeding) reduces the varroa population rapidly and helps secure healthy winter bees. A long-term treatment after feeding is applied to kill mites that survived the short-term treatment as well as reinvading mites.

Due to different parameters, such as temperature, there is sometimes a large variation in efficacy, and bees may be damaged and/or queens may be lost under some conditions. Treatment with formic acid is relatively inexpensive, but requires experienced beekeepers that can alter the dose and form of application depending on the situation. Formic acid treatment has the advantage that there is an effect also on mites in the sealed brood cells.

### Lactic acid

Lactic acid kills mites on adult bees only. It is sprayed directly on the bees in broodless colonies, and is used mainly in the fall after feeding. To secure a low mite level in wintered colo-nies, treatment with lactic acid is recommended when a high mite drop during the long-term formic acid treatment appears. With lactic acid there is little (or no) risk of damages to bees, and it is a relatively inexpensive, however labour intensive, method. Research has shown that two to three treatments in the fall combined with drone brood removal in spring/early summer can control varroa under Swedish and Danish conditions. Such a concept is feasible for beekeepers that do not run large number of colonies and who do not want to use formic acid.

### Nuclei/splits

By forming nuclei/splits during spring and summer varroa population can be reduced in the mother colonies. The nucs produce new colonies that can replace winter losses. Requeening is quick and easy, and the nucs can be easily treated for varroa in the absence of brood. Nuc formation incorporated into the general management system may be suitable for larger operations. However it requires skilled beekeepers and also some surplus material.

### Trapping comb

By applying the trapping comb system during the main nectar flow in the summer, the varroa population can be reduced considerably. Trapping comb is recommended when the infestation level is high already early in the season and if the beekeeper still wants the colonies to produce honey and be suitable to winter. The queen is trapped on one or two frames and worker comb as well as drone comb can be used. The sealed worker combs produced are suitable for nuclei formation after either heat-treatment or treatment with formic acid. Research has shown that the trapping comb technique can control varroa under Swedish conditions if combined with drone brood removal. Such a concept is feasible for beekeepers that want to avoid any chemical substances in their beekeeping (in this case brood combs are not treated with formic acid). The trapping comb system requires skilled beekeepers and is labour intensive. It also requires a queen in the colony and one queen only, and is not suitable for late flows.

## Oxalic acid

Oxalic acid kills mites on adult bees only. It can substitute lactic acid as a treatment for broodless colonies. There are two different application methods: sprayed directly on the bees (labour intensive) or trickled onto the colonies on the populated bee spaces (quick and simple). Oxalic acid treatment is effective and relatively inexpensive, but bee tolerability is low under some circumstances. Data on bee tolerability and optimal dose is needed before oxalic acid application can be recommended to beekeepers in northern climates if treatment is applied in late fall.

## Evaluation of mite mortality

Evaluation of the natural mite mortality in early summer and of the mite mortality during (and/or after) treatment in the fall is needed in most cases. Based on mite mortality data, decisions can be made if further control measures are needed.

## Basic treatment concept

Figure 1 shows the basic concept that is now widely recommended in the Nordic countries: drone brood removal in spring/early summer, evaluation of natural mite mortality in June, treating once or twice - depending on infestation level - with formic acid after honey harvest and finally treating with lactic acid after feeding - if mite drop during previous treatment is high.

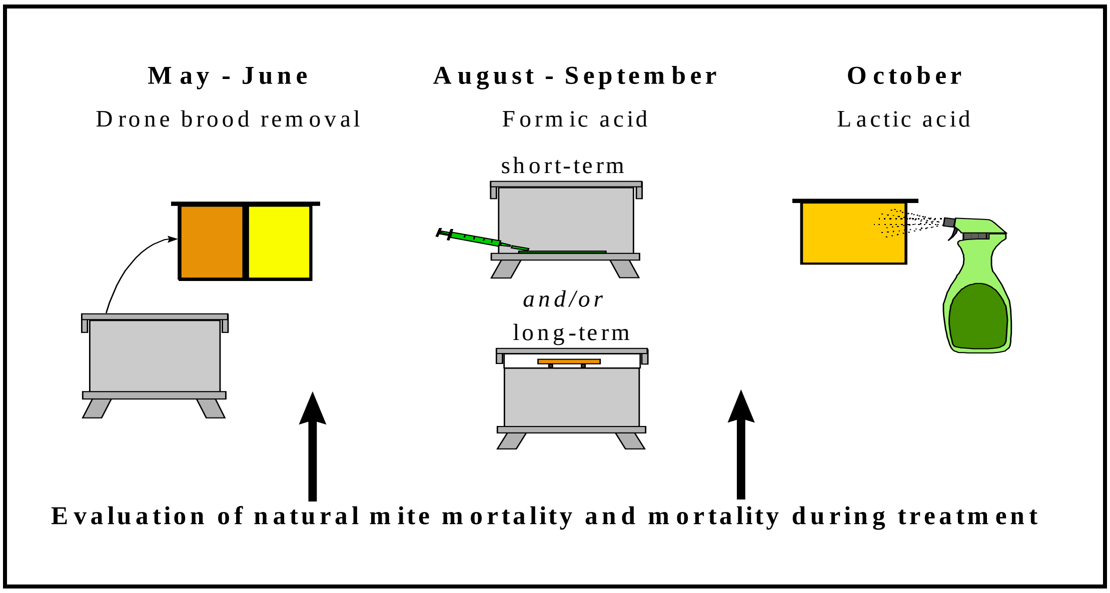


Figure 1. Basic concept recommended in the Nordic countries for control of varroa

This concept is relatively unproblematic in areas with summerflow only, especially for hobby beekeepers. In small operations, time is usually not the main problem and therefore there are good ecological control alternatives available. However in areas with a late flow, where the period between honey harvest and winter is short, this concept is more problematic especially for beekeepers with many hives.

Thus, it is important to develop application methods and concepts that are sufficiently effective even for areas with late flows, and which are less time consuming than the methods so far available. Development of safe and effective methods for trickling of oxalic acid would help in this respect. Evaluation of the different application systems for formic acid treatment in order to optimise treatment, reduce damages to bees, variation in effect, and the time needed for application, should also be studied. Essential oils and possibly even heat-treatment of sealed brood are other methods that could be of interest in integrated control of varroa in North Europe.

## TREATMENT CONCEPTS THAT CAN BE RECOMMENDED TODAY FOR NORTH EUROPE

### Treatments for varroa control in colonies that are used for summer honeyflow in small and medium sized apiaries

Concept A

- drone brood removal in spring/early summer (two to three combs)
- monitoring natural mite mortality in June
- nuclei formation, with some treatment taking advantage of broodless conditions such as introducing and removing drone brood or lactic acid treatment
- short-term treatment with formic acid right after honey harvest
- long-term treatment with formic acid after feeding
- monitoring mite mortality during formic acid treatment
- lactic acid treatment if necessary (more than 300 mites from last formic acid treatment). Repeat treatment until less than 50-100 mites fall from last treatment)

Concept B

- drone brood removal in spring/early summer (two to three combs)
- monitoring natural mite mortality in June

- nuclei formation, with some treatment taking advantage of broodless conditions such as introducing and removing drone brood or lactic acid treatment
- lactic acid treatment with monitoring of mite mortality. Repeat treatment until less than 50-100 mites fall from last treatment

Concept C

- drone brood removal in spring/early summer (two to three combs)
- monitoring natural mite mortality in June
- nuclei formation, with some treatment taking advantage of broodless conditions such as introducing and removing drone brood or lactic acid treatment
- short-term treatment with formic acid right after honey harvest
- lactic acid treatment with monitoring of mite mortality. Repeat treatment until less than 50-100 mites fall from last treatment

Concept D

- drone brood removal in spring/early summer (two to three combs)
- monitoring natural mite mortality in June
- trapping comb in summer during main flow (trapping combs used for nuclei formation after formic acid treatment if worker comb is used, drone comb is destroyed)
- monitoring natural mite mortality in fall
- lactic acid treatment if necessary (more than 1 mites per day - natural mortality).

Repeat treatment until less than 50-100 mites fall from last treatment)

### Treatments for Varroa control in colonies that are used for late honey flow in small and medium sized apiaries

Concept B above and E below (suitable if infestation rate is low)

## POSSIBLE TREATMENT CONCEPTS THAT NEED MORE RESEARCH BEFORE RECOMMENDED FOR NORTH EUROPE

### Treatments for Varroa control in colonies that are used for late honey flow in small and medium sized apiaries

Treatment A above and E below.
*The risk of contaminating the late honey harvest with the first formic acid treatment needs to be evaluated.*

## Treatments suitable for large scale operations (also suitable for small and medium sized apiaries)

Concept E

- drone brood removal in spring/summer (preferably two to three combs)
- monitoring natural mite mortality in June
- nuclei formation, with some treatment taking advantage of broodless conditions such as introducing and removing drone brood or lactic acid treatment
- long-term treatment with formic acid after honey harvest and feeding

*We lack hard data that this combination of treatments gives sufficient control*

Concept F

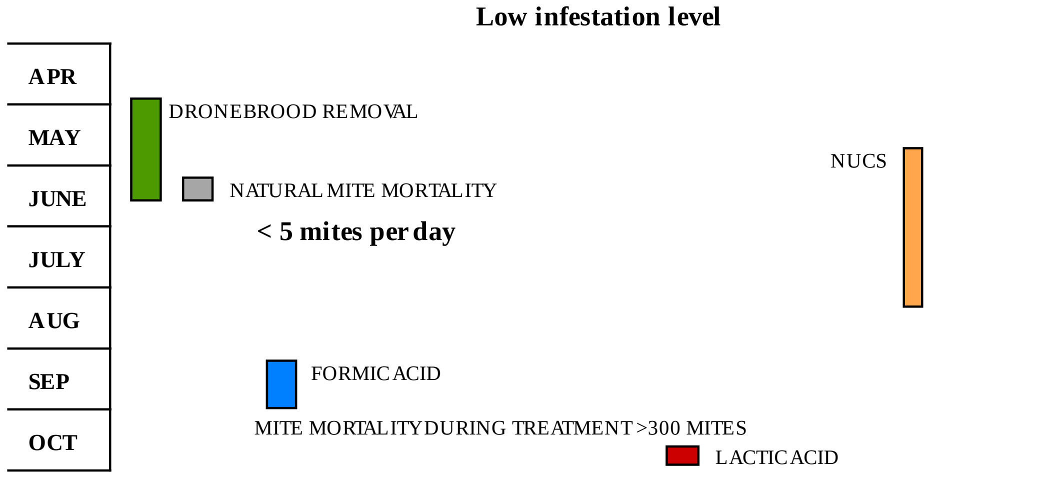
- drone brood removal in spring/summer (preferably two to three combs)
- monitoring natural mite mortality in June
- nuclei formation, with some treatment taking advantage of broodless conditions such as introducing and removing drone brood or lactic acid treatment
- oxalic acid trickling in fall

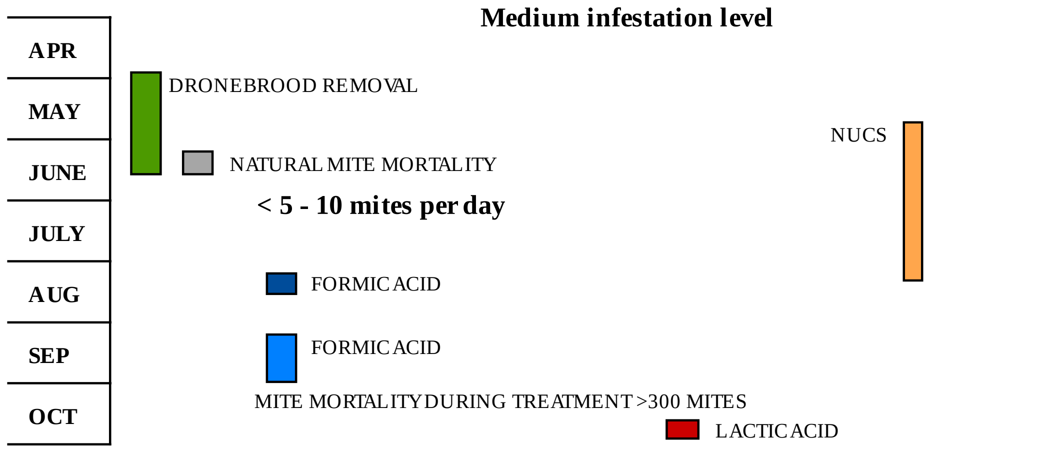
*Dose and bee tolerability of oxalic acid trickling needs further evaluation.*

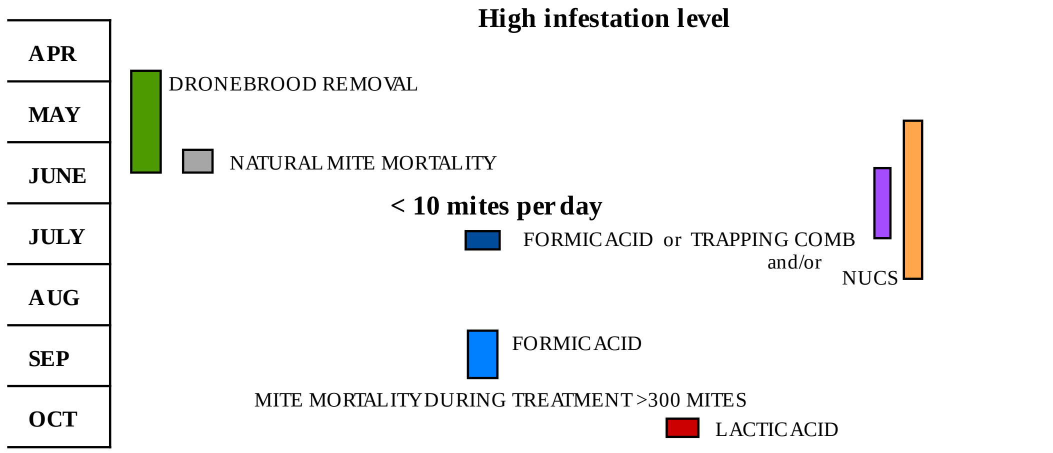
Concept G (requires three separate apiaries)

- formation of nuclei (adult bees + queen) and replacement of colonies in a rotation system using three types of colonies. 1. first year nuclei; 2. second year production colonies 3. third year production plus nuclei mother colonies
- treatment of nuclei (adult bees only) with oxalic acid trickling
- wintering of nuclei and second year colonies only

# Control concepts based on mite mortality



# Ecological Varroa Control

notes on control strategies for Central Europe

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## Introduction

The bee parasite *Varroa jacobsoni* has become resistant to various conventional varroacides in many regions of Europe. Alternative varroa control is a strategy that may reduce the problems associated with mite resistance to acaricides and avoid that mite control develops into a dead-end. Alternative substances such as organic acids and constituents of essential oils are available as substitutes. The application of these alternative active substances is only successful, however, if they are used in a treatment strategy.

We present in this article such treatment concepts, which have been developed and successfully tested in extensive investigation under Central European conditions during the last ten years.

## Varroa control strategy

Monitoring the varroa population is an important measure, because only then an increase in the mite population is recognised early and the necessary control measures can be introduced before colonies are actually damaged. The most important part of the control strategy is the reduction of the mite population in August and September after the honey harvest by one or two treatments with formic acid or with thymol. As soon as the colonies are free from brood, they are treated again in November with oxalic acid or with Perizin. When this concept is carried out consistently, further treatments before the end of the honey harvest of the following year are not necessary.

## Monitoring the *V. jacobsoni* population

By means of mesh-protected inserts, rapid increases of infestation through reinvasion or insufficient treatment success can be recognised early and necessary control measures taken in time.

When resistant mites increase, there is a danger that the damage threshold will be exceeded sometime and colonies will collapse. This in turn can lead to massive re-invasions of neighbouring apiaries. It is therefore necessary to monitor mite fall from time to time between spring and the end of July. If mite fall is higher that 30 per day, control measures must be initiated, irrespective of time. With a weekly treatment using formic acid for example, dying of colonies can be avoided.

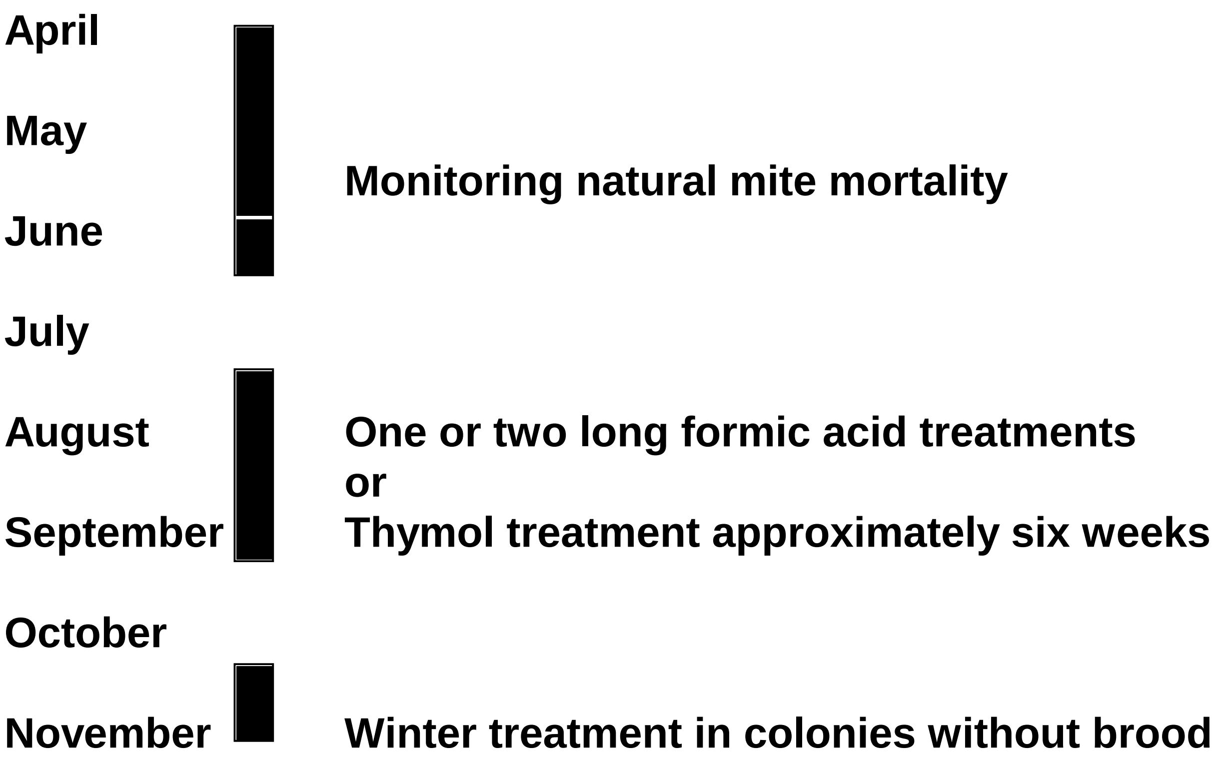


Figure 1: Alternative varroa control is only successful if used as a strategy. Monitoring the degree of infestation and reducing the mite population in August and September with formic acid or thymol and in November with oxalic acid or Perizin are the corner stones of this strategy. Reducing the varroa population in spring by biotechnical measures (drone removal) is also recommended.

*Table 2: Interpretation of the natural mite fall.*

| Time | Mites per day more than | Measures |
|---|---|---|
| End of May | 3 | A long-term treatment with formic acid should be carried out immediately after the spring harvest. |
| End of July | 10 | Two long-term treatments with formic acid are necessary. |
| Beginning of September | 1 | A second long-term treatment is necessary. |
| Entire bee season | 30 | Damage threshold reached. Colony collapse imminent. Treatment without delay is imperative. |

## Reduction of the mite population in spring

If mite fall at the end of May exceeds 3 varroa per day, treatment can not wait until August. In the next gap between honey flows, a long-term treatment of one week, or two short-term treatments, with formic acid should be carried out in colonies with their supers removed. Treatments between honey flows often cause formic acid residues from the treatments in a subsequent honey harvest. These treatments are therefore to be used in emergencies only.

The varroa population can be reduced by about half by cutting out drone brood twice or three times, or by about a third by forming a nucleus. It is an advantage to integrate such measures into routine beekeeping procedures.

## Formic acid treatment after the end of the honey flow

Various types of dosing equipment for long-term treatment with formic acid are available on the market. Since *V. jacobsoni* control is completed later on with a winter treatment, it is not essential to achieve the highest possible treatment success with formic acid. Thereby the risk of queen losses is considerably lowered. Application of formic acid using one of the numerous dispensers must be in accordance with instructions for that specific device.

## One or two treatments with formic acid?

Why treat twice if once is enough? Each treatment represents a stress for the colony, it is therefore important to reduce number of treatments to a minimum.

If the natural mite mortality is above 10 mites per day at the beginning of August, two long-term treatments are needed; the first should be done immediately after the honey harvest. The second treatment should be from mid-September. For a mite fall of less than 10 per day one treatment is sufficient. This treatment can be postponed until the end of August.

The necessity of a second formic acid application can also be checked by means of the natural mite mortality during the third week after the end of the first treatment. If the number of mites that fall are above one varroa per day, a second application should be carried out.

Effectiveness of one treatment can be expected to be between 60 and 80%. Over two applications, the effectiveness increases to 90-95%. Formic acid is effective in capped brood cells and also kills tracheal mites (*Acarapis woodi*) inside the trachea.

## Treatment with components of essential oils

Instead of formic acid, treatments with thymol can be carried out. The market offers several products where the effective ingredient thymol is applied or incorporated into different carrier substances. For evaporating thymol, these products are placed on the top bars over several weeks.

The application should always be according to the instructions of the manufacturer. After the honey harvest is completed, colonies should be fed as much food as possible. As with formic acid, treatment has to start as early as possible when the natural mite mortality exceeds 10 mites per day. With products where the first dose is replaced by a second dose after 2-3 weeks, feeding is beneficial before application of the second dose.

It is estimated that effectiveness from thymol treatments under optimum conditions are between 90 and 97% (Imdorf 1999). Monitoring treatment success is not necessary because of the subsequent application of oxalic acid in November.

## Winter treatment in broodless colonies

Treatment with oxalic acid or Perizin in November aims to reduce the residual mite population to a minimum. If this is achieved and only few mites winter with the bees, and provided there is no reinvasion of mites in the spring, further control measures can normally wait until August the following year. Reducing the mite population to such an extent that treatment can wait until the following August can only be achieved when colonies are treated without sealed brood present in the colonies. Oxalic acid and Perizin have no effect on mites in sealed brood. The need for a winter treatment with oxalic acid or Perizin can be checked by counting the natural mite mortality in October. If the mite mortality exceeds a limit of about 3 mites per week, then the winter treatment is recommended.

## Spraying oxalic acid

To spray oxalic acid, a solution of 30 g oxalic acid dihydrate to 1000-ml water is used. Three to four ml solution is sprayed on each side of the comb occupied with bees using a hand sprayer. This method is well tolerated by the bees and is particularly suitable for bee colonies in single brood chamber. Since every comb need to be lifted and each comb side with bees need to be treated, the application is time consuming.

## Trickling oxalic acid

For trickling oxalic acid, a solution of oxalic acid dihydrate in sugar water (35 g OA dihydrate in 1 litre sugar water 1:1) is used. Five ml of this solution per space between combs occupied by bee, is dripped (trickled) on the bees. Depending on colony size, 30-50 ml per colony is needed. This application is less labour intensive compared to spraying oxalic acid; however, it is less well tolerated by the bees. Formulation, concentration, and dosage for trickling oxalic acid must be further improved before it can be recommended to beekeepers in Central Europe. Therefore, the spray method should be preferred if oxalic acid is used until more data on bee tolerance and efficacy on the mite is available for various doses and concentrations .

Repeated applications of oxalic acid by trickling are not recommended, due to the low bee tolerance. Thus, if colonies have sealed brood oxalic acid trickling is not an effective method since it should not be repeated.

Both trickling and spraying oxalic acid have an effectiveness of over 95% provided the colonies are free from brood.

## Treatment with Perizin

The Perizin solution is trickled in the space between combs occupied by bee as for oxalic acid trickling. The treatment has to be performed according to instructions of the manufacturer. In most cases, however, a single treatment is enough if the treatment strategy described above has been adopted.

## Protective measures

During application of organic acids, essential oils or chemicals, acid-proof gloves must always be worn. When handling formic- and oxalic acid, protective goggles should also be worn and a bucket of water placed within reach. When spraying oxalic acid solution, a breathing mask of the type FFP2SL, EN 149 must be worn. Oxalic acid solutions should only be made by a suitably qualified persons.

## Concluding remarks

This control concept provides a means for the beekeeper to keep varroa mites below the damage threshold in most regions of Central Europe. With relatively low labour investment, it still remains possible to successfully produce high quality bee products also in the future.

Some products mentioned in this text are not registered in some Central European countries. Beekeepers have to keep informed about what treatments that are legal in their respective country.

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## Résumé

Pour faire face aux problèmes de Varroa résistants aux pyréthrinoïdes et de résidus dans les produits apicoles, plusieurs instituts de recherches ont développé des méthodes de lutte alternative contre *Varroa jacobsoni*. Les produits de traitement utilisés sont entre autres les acides organiques et les composants d'huiles essentielles. Des mesures biotechniques telles que la découpe du couvain de mâles ou la formation de jeunes colonies permettent aussi de réduire de manière significative le nombre de Varroa dans les colonies. Pour appliquer avec succès une lutte alternative contre Varroa, il convient de planifier selon la région, le climat, les miellées ou la conduite des ruches différentes interventions durant l'année en une stratégie de lutte.

Une stratégie de lutte ayant fait ses preuves pour les conditions régnant en Europe Centrale comprend les étapes suivantes: au mois d'août - septembre, la population de

Varroa est réduite d'environ 80 à 90% après la miellée en traitant une à deux fois à l'acide formique longue durée ou au thymol durant 4 à 6 semaines. Dès que les colonies n'ont plus de couvain en novembre, on effectue un traitement soit à l'acide oxalique ou au Perizin. Ce traitement permet d'éliminer les Varroas ayant survécus aux traitements estivaux et ceux provenant de la réinfestation. Si cette stratégie est appliquée correctement, il n'est pas nécessaire d'effectuer d'autres traitements jusqu'au mois d'août de l'année suivante. Cependant, en raison du risque de réinfestations provenant de ruchers voisins, il est conseillé de contrôler à certains moment le taux d'infestation des colonies en comptant la chute naturelle d'acariens. Une forte infestation peut ainsi être déceler suffisamment tôt et les mesures de lutte qui s'imposent peuvent être appliquées à temps. Des mesures biotechniques au printemps peuvent également être intégrées dans cette stratégie de lutte.

# Ecological Varroa Control

Additional comments on control strategies for Central Europe with special reference to the situation in Germany

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The general requirements for an effective varroa control strategy in Central Europe include monitoring of the natural mite mortality, summer treatment with formic acid or etheric oils and winter treatment in brood free colonies as outlined by *Charrière & Imdorf* on Control Concepts for Central Europe. From our experience in South Germany over more than 10 years, we would make the following additional remarks:

## Drone brood removal

The removal of 3-4 sealed drone brood combs per colony and season is a crucial point of an integrated varroa control strategy. It is the only useful and effective method to reduce the mite population during the spring. The result is a significantly lower increase of the varroa population which reduces the number of mites at the end of the season to levels of 50 to 75% compared to colonies where drone brood has not been removed. Therefore, drone brood removal reduces the risk of mite damages during the season, in particular in regions with a late honeydew flow, where the summer treatments can not be performed before August/ September.

## Short-term treatments with formic acid

It is very important to treat colonies immediately after the last honey yield. The aim of this first treatment is (i) to reduce the mite population to levels which allow the production of healthy winter bees and (ii) to prevent distribution of mites between different bee yards through robbing or early collapse of colonies. In regions without late honey dew flows the first treatment could be performed during the second half of July. For such early treatments, formic acid short-term treatments have some advantages compared to the long-term treatment devices:

Some short-term methods like "Illertissener Milbenplatte" or "Schwammtuch" do not require additional bee hive equipment.

- The effectiveness and the side effects of formic acid treatments are strongly influenced by climatic conditions (i.e. ambient temperature). Over long-term treatment periods of two weeks and more, the weather condition can hardly be predicted. During the hot summer months long-term treatments cause a higher risk of severe brood damages (or queen losses). In the autumn, a minimum ambient temperature of 15°C, which is a prerequisite for an effective evaporation of formic acid, is only reached on single days. Short-term treatments (evaporation over a 24 – 36 hours period), however, can easily be applied in mid summer according to the actual weather condition.

For short-term treatments, we recommend two methods:

1. "Schwammtuch" with 60% and 85% FA (20–60ml) depending on ambient condition and application from above or below.
   "Medizinflasche" with a special wick and 85% FA (20-50ml) according to colony strength.

# Ecological Varroa Control

notes on control strategies for South Europe

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## Introduction

To understand the present situation of apiculture in Southern Europe in general and in Spain in particular there are some characteristics that need to be considered. Many professional beekeepers run large apiaries (500-2000 hives or more) mainly using Layen hives (in Spain) and do migratory beekeeping. Hobby beekeeping (5-50 hives) using mainly Langstroth hives is also widely spread. Furthermore, the climate in this part of Europe results in broodrearing throughout the year and, thus, in continuous reproduction opportunities for varroa (*Varroa jacobsoni*) mites.

The structure of apiculture and the climatic conditions for beekeeping require that several different means for control of varroa mites are available. Control methods employed need to be adapted to both the variations in beekeeping methods and apiary sizes as well as to the continous presence of brood. In this document we want to propose a number of control methods which in combination will allow beekeeping without traditional acaricides, or at least minimize its use, and still maintain viable and productive honey bee colonies.

In order to obtain sufficient efficacy from the control methods proposed below, the following needs to be considered:

The treatments should be **coordinated** in each geographical area, the application methods and their timing should coincide. Coordinated treatments are needed to minimize the important problem of mite reinfestation into already treated hives.

Proposed treatments must be **simple, cheap**, **quick** to apply, and sufficiently **efficient** in order to be adapted by the majority of beekeepers. These aspects are even more important for the professional beekeepers.

Because of continuous presence of bee brood, no single treatment can be expected to give sufficient control of the mites. Thus, repeated treatments are needed, emphasizing the need for use of products with low toxicity to the bees and with low residual problems for the hive products.

## Main treatments

Based on the experience from clinical trials from South Europe, it is clear that varroa mites can be controlled without use of traditional acaricides (fluvalinat, amitraz, etc). However, the application of alternatives to these acaricides often increases the need for management of the hives, a substantial disadvantage especially for professional beekeepers.

Some treatments that offer the best alternatives to traditional acaricides are presented below:

***Api Life VAR.***

**Purpose**:

To kill mites after honey harvest in order to secure healthy winter bees.

**Methodology:**

**a)** low dosage (1 tab broken into 3-4 pieces/week administered 3 times)

**b)** full dosage (2 tab broken into 3-4 pieces for 12 days, then repeat the same treatment one more time).

**Time of treatment**:

a) and b):

Immediately after removing the last summer honey. In order to obtain acceptable efficacy it is indispensable that temperature is adequate (maximum day temperature around 20-25ºC) and that there is some activity in the treated bee colonies .

**Main advantages**:

a) and b):

Simple administration.

a) containment of side-effects, treatment-cost reduction.

**Main disadvantages**:

a) and b):

Some variation in efficacy due to different parameters (weather, colony condition, model of hive, etc.).

Low effectiveness in Layens hive.

Loss of active ingredient during storage.

In strong hives the product is quickly removed by bees with negative effects on efficacy.

b) damages to bees and brood under some conditions (high temperature)

risk of robbing at high temperatures

risk of absconding at high temperatures.

The presence of brood in the spring drastically reduces effectiveness.

***Thymol (powdered crystal).***

**Purpose**:

To kill the mites in early spring and early autumn in order to secure healthy honeybees colonies.

**Methodology:**

8 g/hive on the top bar of the combs. Repeated administration 3-4 times at 1-week interval.

**Time of treatment**:

In early spring (March) with mean daily temperatures of 10-15ºC. After the last honey removal in summer (October-November with mean daily temperatures of 10-15ºC).

**Main advantages**:

Effective, cheap and simple treatment.

**Main disadvantages:**

Some variations in effectiveness under sub-optimal conditions.

Damages to bees and brood under some conditions (high temperatures).

Risk of queenloss (high temperatures)

Risk of hive abandonment (high temperatures)

Application method may result complicated in Layens hive.

***Oxalic acid.***

**Purpose**:

To kill mites in broodless colonies.

**Methodology**:

a) trickling application (sugar solution).

10:100:100 solution (gram oxalic acid dihydrate: gram sugar: ml water)

5 ml/ between comb spaces occupied by the bees.

1 administration.

**b)** spraying directly on the bees (in water only).

3:100 solution (gram oxalic acid dihydrate: ml water)

3-4 ml/comb side occupied by the bees

1 administration.

**Time of treatment**:

Optimal in broodless colonies.

**Main advantages**:

a) Feasible in large apiaries. Simple, effective and cheap treatment

b) Feasible in small apiaries (up to 50 hives). Effective and cheap treatment.

**Main disadvantages**:

a) and b):

Problems with bee tolerability have been reported.

The presence of sealed brood drastically reduces the efficacy.

In certain geographical areas (central part of Spain) only one application may not be sufficient to guarantee good acaricide efficiency (≥ 95%), even if colonies are broodless during treatment.

a) The optimal concentration of oxalic acid as well as the sugar concentration is still being investigated.

Some hive constructions make treatment more difficult (Layens hive).

In large operations (≥ 200 hives) the time needed for treatment may be to long.

b) The treatment (sparaying) is not advised in apiaries with more than 50 hives, because of the time required to carry out the treatment.

## Complimentary techniques

The treatments described below are intended as a complement to the previous treatments in a program of integrated varroa mite control. As exceptions, skilled beekeepers with limited number of hives, may be capable of substituting some "main treatments" with some "complementary techniques".

### *Formic acid.*

**Purpose:**

To kill the mites in early spring and early autumn in order to secure healthy honey bee colonies.

**Methodology:**

Treatment between harvests, or preferably after the last honey harvest.

Dosage and timing of treatments:

40 ml 85% formic acid soaked pad inserted in the lower part of the hive ("treatment from belowÓ). Repeat administration 4 times at 1-week intervals. This treatment is suggested for mid-August or later.

30 ml 60% formic acid soaked pad placed on the top bar of the frames ("treatment from above"). Repeat administration 4 times at 1-week intervals. This treatment is suggested for early summer.

Nassenheiderverdünnster: 180 ml 60% formic acid placed in the middle of the hive. Repeat administration 2 times at 14-days interval. This treatment is suggested for springtime.

ER.FROM: 30 ml of 85% formic acid placed on the top of the hive. Repeat administration 4 times at 1-week interval. This treatment is suggested for autumn.

**Main advantages:**

Cheap treatment.

Effective also on mites in sealed brood.

**Main disadvantages:**

Some variations in efficacy due to different parameters (weather, colony strength, etc.). Large variation in efficacy under South European conditions. (30-95%).

Variable in efficacy even in hives within the same apiary.

Damages to bees and brood in hot climates.

Risk of queen losses in hot climates.

Risk of absconding in hot climates.

Time consuming applications

Requires skilled beekeepers

***Drone brood removal.***

**Purpose**:

To reduce the growth rate of mite populations during the active season.

**Methodology:**

Drone brood reared in special combs which are periodically removed and destroyed.

**Time of treatment:**

Spring-early summer.

**Main advantages:**

Reduced mite populations before summer treatment can start.

**Main disadvantages:**

Time consuming.

The schedule has to be strictly followed.

Difficult to carry out in large operations (≥ 50 hives).

## Conclusion

Field experience demonstrates that the treatments mentioned above can be used to maintain varroa populations below the level where colonies become damaged. However, some of the application methods are laborious. It is necessary to invest time in implementation as well as using several visits to the apiary for some applications. This causes an increased production costs for beekeepers, of particular importance for professional beekeepers that manage hundreds or even thousands of hives.

The presence of sealed brood will largely determine the efficacy of some of the control treatments available. Different timing of nectar supply, colony development and honey harvest period will influence when treatment of bee colonies is possible or appropriate. Thus, detailed information on the climatic and foraging conditions is needed to make a functioning plan for an optimal treatment strategy. In this sense, it is inevitable that suggestions for control of varroa mites in honey bee colonies should be extraordinarily “regionalized”.

Although it is possible to gain sufficient control of varroa using existing alternative methods, there is still a great need to improve application methods (for example thymol) or to optimize the dosis to increase bee tolerability (for example oxalic acid). There is also need to further study the residue situation in bee products when alternative methods are used. With the increased awareness of mite tolerance to products used for mite control, it is fundamental to have a wide range of options which allows sufficient control of the mite. An integrated approach where alternative products are included can reduce the total input of traditional acaricides, decrease residue problems and lower the risks of building up acaricide resistance in the mite population