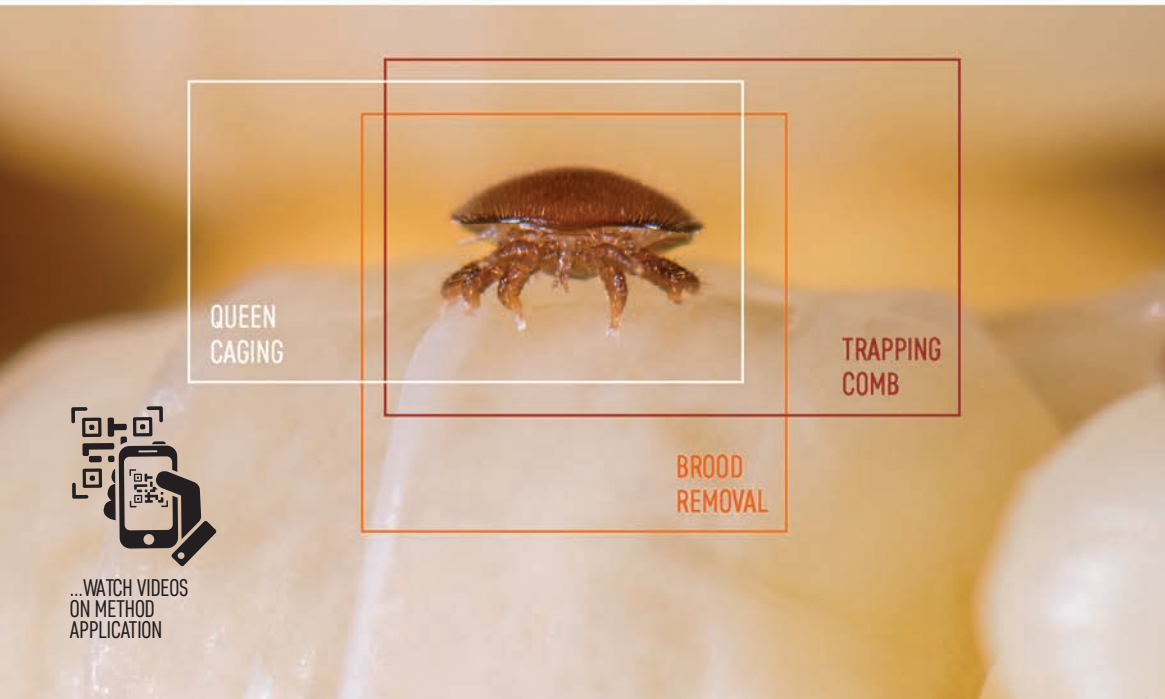
ALEKSANDAR UZUNOV
RALPH BÜCHLER

ALTERNATIVE METHODS FOR VARROA CONTROL IN HONEY BEE COLONIES



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ALTERNATIVE METHODS FOR VARROA CONTROL IN HONEY BEE COLONIES

... WATCH VIDEOS
ON METHOD
APPLICATION

The handbook "Alternative methods for Varroa control in honey bee colonies" is developed as part of the Nature Conservation Programme in North Macedonia – phase 2 (NCP), a project of the Swiss Agency for Development and Cooperation (SDC), coordinated by Farmahem-Skopje.

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NATURE CONSERVATION PROGRAMME

ФАРМАХЕМ
FARMAHEM

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TABLE OF CONTENTS

PREFACE

The Nature Conservation Programme in North Macedonia aims to help the country to effectively preserve the natural values in the Bregalnica region, by the integration of modern approaches to nature conservation with the principles of sustainable management and use of natural resources. In this context, the programme pays particular attention to supporting the activities for improving beekeeping practices in the Bregalnica region in order to have safe and good-quality bee products, as well as protect beekeepers and the environment. Thus, one of the numerous activities was the implementation of the applicative-research project "Alternative approach for *Varroa destructor* control in the summer period", which tested and promoted the methods for controlling *V. destructor* in the summer period without the use of commonly applied synthetic chemicals.

This handbook is the final product of the project, and its objective is to educate beekeepers, straightforwardly, about the methods' application and at the same time, give a visual presentation of the procedures. That is why the methods in the handbook are explained "step by step", while in an avant-garde way for beekeeping literature the methods are documented with videos, which are immediately accessible for readers via QR (Quick Response) code.

Partners in this project were the members of the core group and beekeeping association Meden Istok in the Bregalnica regions, Kochani, (CG Meden Istok) in whose apiaries the research and the methods were implemented in the period between 2017 and 2019, for which we express gratitude to Ilija Litajkovski, Goce Ruzhinski, Mirjana Ivanovska, Nenad Janchov, Sasho Spasov, Nada Jangelovska, Miki Janchov and Natasha Velkovska. Special thanks to the president of GC Meden Istok association, Vancho Kirovski, for his dedication to coordinating and implementing the activities in the field.

For the support in the project implementation and the suggestions for improving the handbook, we would also like to thank beekeepers MSc Borche Pavlov, Branko Sokolov, Goran Jakimov Martin Gabel, Ilija Petrovski, Jonche Dodevski, as well as the students at the Faculty of Agricultural Sciences and Food in Skopje, Blagoja Dolgovski, Martina Deskovska and Monika Velkovska.

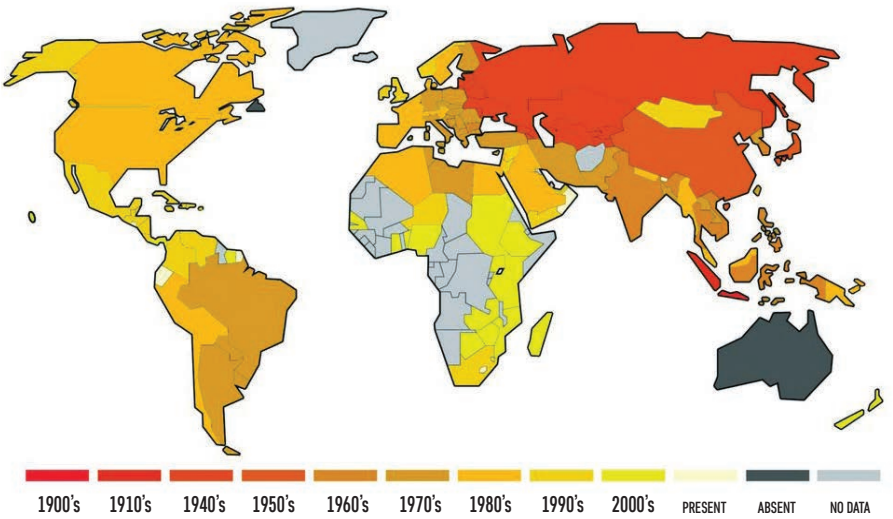
We sincerely hope that with this handbook we will encourage beekeepers to apply the described methods and improve the traditional concept for controlling *V. destructor* in honey bee colonies, thus contributing to the production of safe and good-quality bee products. Finally, having in mind the experience with our cooperation so far, we expect ideas and proposals from the beekeepers about improving the methods and successfully integrating them in beekeeping practices, for which we are thankful in advance.

Nature Conservation Programme in North Macedonia, Skopje, 2020

INTRODUCTION

...to watch videos

By shifting from the Eastern honey bee (*Apis cerana*) to the Western honey bee (*Apis mellifera*), ectoparasite *Varroa destructor*[1] spread globally in a short period of time (Figure 1), causing significant losses of honey bee colonies and economic damages to the overall beekeeping industry.



1 The global spread of *V. destructor*, per decades (modified according to Wilfert *et al.*, 2016).

In certain European countries, as part of the COLOSS[2] network, there is a monitoring of the losses of honey bee colonies during winter, and the registered average losses are between 10% and 30%. These losses of colonies are often linked to the impact of the *V. destructor* mite and the success of the treatments applied to control it. In any case, high infestation with mites in the period of rearing "winter" bees, i.e. in the second half of summer, is a strong risk factor when it comes to the overwintering of honey bee colonies.

In North Macedonia, similar to the trends in other European countries, in the last decade, there have been registered winter losses of honey bee colonies of 8% (2015-2016.) to almost 31% (2011-2012.). The high infestation with *V. destructor* is one of the main factors for those occasional losses in some regions in Europe and the world. Indeed, when "dissecting" the reasons for losses of honey bee colonies one should not exclude or minimize the negative impact of other stress factors, including other bee pathogens, pesticides, monocultures, poor beekeeping practices, etc.

[1] Class *Arachnida*, Subclass *Acari*, Superorder *Parasitiformes*, Order *Mesostigmata*, Family *Varroidae*, Genus *Varroa*.
[2] COLOSS - Prevention of honey bee COlony LOSSes (www.coloss.org).

If the mite infestation is not monitored and controlled, it is expected that the majority of the honey bee colonies would collapse within 2 to 3 years. However, the negative consequences of non-implementation or untimely implementation of the treatment can be noticed even in the first year, when the health of the colonies deteriorates, and colonies usually do not achieve the expected results the following year. This situation is also due to the damaging impact of bee viruses, to some of which the mite is a vector.

In the first attempts to control the mite, beekeepers and experts were mostly focused on developing and applying methods with the use of synthetic chemicals. In this way, they tried to quickly and efficiently control the parasite and prevent mass vanishing of honey bee colonies. However, the use of synthetic chemicals (flumethrin, amitraz, coumaphos, tau-fluvalinate, etc.), especially when the recommendations for proper application are disregarded, might lead to the presence of residues in honey bee products. This approach is a risk for the safety of the products, the users, the beekeepers and the environment, and might make the mite resistant to synthetic chemicals.
On the other hand, by better understanding the mite's biology, and in order to avoid the negative consequences of the synthetic chemicals used to control the mite, there is increased interest in developing and implementing so-called bio-technical methods. These methods are based on the use of certain aspects of the biology of honey bees and mites and their relationship, with the application of specific methods in managing and manipulating the honey bee colony to control the population of mites without the use of chemicals. There are numerous bio-technical methods and combinations thereof, many of which are recommended and applied in organic beekeeping. Bio-technical methods include:

- screened bottom board
- drone comb (trap)
- caging or trapping the queen bee
- brood removal
- selection of *Varroa*- resistant honey bee populations
- Use of ultrasound, temperature, smaller comb cells, rotation of comb with brood, etc.

Some of these methods or combinations thereof are proven to have a significant positive impact on mite control. On the other hand, not all methods are easily applicable or accepted by beekeepers.
One group of biotechnical methods, called methods for brood interruption[3] in summer (brood removal, queen caging and trapping comb) are particularly interesting for beekeepers because of their applicability and efficiency in mite control. These methods, combined with the use of drone brood trap in spring and particularly with the application of winter treatment with oxalic acid, are regularly used by some beekeepers in various regions in Europe. In this way, the mite can be controlled with only 2 treatments per year: one summer treatment during or close to the end of the main foraging, and in late autumn or early winter when no brood is expected in the colonies. This concept of mite control without the use of synthetic chemicals is the basis for improving the safety of bee products, the safety of beekeepers, for protection of the environment and better economic results from beekeeping production.

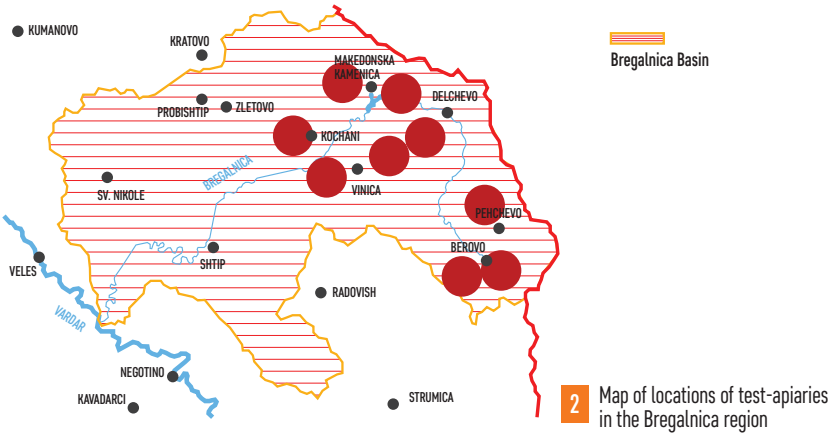
In the conditions in our country, particularly in the regions under the influence of Mediterranean climate, the season of brood rearing is prolonged, which is an opportunity for the development of the mite population in the honey bee colonies. On the other hand, this is an excellent opportunity for application of some methods for brood interruption in the summer period because the colonies, after some of the

[3] The term "brood interruption" refers to the methods with which brood rearing is interrupted or restricted in honey bee colony.

methods are applied, will have sufficient time to recover for the risky winter period. In any case, the success of the applied methods is dependent on various aspects, primarily the environmental conditions, then the intensity (type) of beekeeping (hobby, additional activity or commercial beekeeping), as well as the beekeeping experience .

In order to test the application and the efficiency of the methods for brood interruption in summer, as an alternative to traditionally used synthetic chemicals, we carried out a two-year research (2017-2019) in 9 apiaries in the Bregalnica region (Figure 2).

The research was conducted under the usual beekeeping conditions when the following methods were tested: "queen caging" and "brood removal", which were compared to the method of applying amitraz with fumigation.



2 Map of locations of test-apiaries in the Bregalnica region

This research, as well as the practical and research experience with these methods in various regions in Europe, are the basis for drafting this handbook, which describes the biological bases of the methods, their application, obtained results as well as recommendations for successful method application.

BIOLOGICAL BASES FOR METHOD APPLICATION

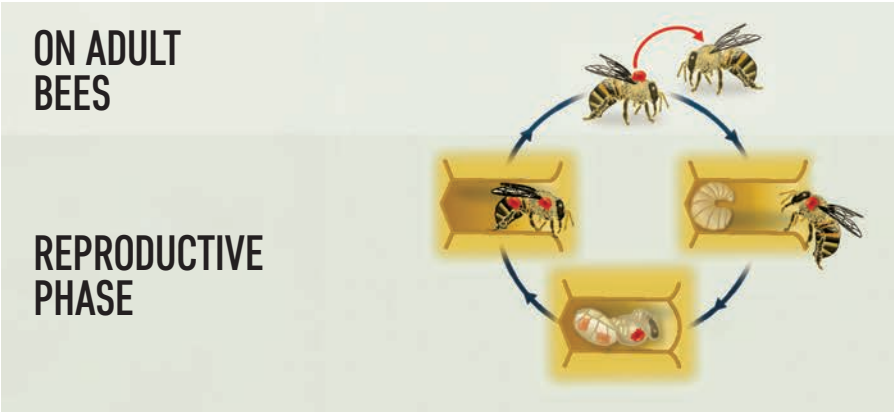
From an evolutionary point of view, *V. destructor* is a new parasite on the Western honey bee (*A. mellifera*), because of which this honey bee species has not yet developed a successful level of resistance, as is the case with the original host, *A. cerana*. That is why without treatments for mite control, there are significant losses of *A. mellifera* colonies. However, it should be mentioned that in the *A. mellifera* species, in various subspecies as well as populations that are subject to systematic selection, there are differences when it comes the resistance to *V. destructor.*

There are two phases in the life cycle of female *V. destructor* (Figure 3):

• Reproductive, which takes place in the sealed brood of worker bees and drones, when the mite reproduces and provides a new generation, and

• On adult bees, when the mite is on their body, which allows for it to be transported to a cell with open brood (before it is sealed) so that it can enter a reproductive phase once again.

Additionally, through foraging bees, and mostly when drifting or robbing other honey bee colonies, as well as through the process of swarming, the mite has the opportunity to spread to new colonies and areas.



3 The life cycle of *V. destructor* (simplified presentation, modified according to Nazzi and Le Conte, 2016).

THE MITE PREFERS DRONE INSTEAD OF WORKER BEE BROOD 8 TO 10 TIMES MORE.

The reproductive phase begins when the reproductive female mite enters a cell of open brood (15 to 50 hours before the capping) and ends when the worker bee or the drone emerges together with the foundress-mite and her offspring so that the mite population in the colony increases.

4 *V. destructor* on bees.

5 Bee with deformed wings

From the start of the season, with continuous brood rearing, until the end of summer, the level of infestation might reach or grow beyond 5 mites per 10 g worker bees (≈ 100 worker bees), which is around 5% of infestation, considered a critical threshold.

The high level of infestation (Figure 4) can result in loss of colonies during the forthcoming winter period or can have a significant impact on the colony development in the following season.

According to research carried out in Germany, such losses are the result of mite activity and viral infections (particularly the deformed wing virus – DWV, Figure 5).

This is of additional significance because it is known that the mite has an important role in transmitting various viruses among honey bees. It is important to note that in regions with the continuous and prolonged rearing of brood, the risk of reaching such critical levels in honey bee colonies is higher.

Brood interruption occurs naturally during the swarming of the colonies (Figure 6) when there is an interruption of the mite's reproductive cycle, and its population is split between the swarm and the colony.

However, modern beekeeping presupposes the implementation of systematic control of

6 Swarm of bees on a branch

swarming, which prevents the natural mechanism of brood interruption in the colonies, and at the same time, the mite's reproductive cycle.

On the other hand, the association between swarming and reduction of mite infestation has been used by beekeepers and experts in the development of biotechnical methods for brood interruption in summer and their application in beekeeping practices.

In fact, the methods are based on the utilisation of the mite's and honey bees' biological features. Thus, mites need brood to reproduce, and with the removal of the combs with sealed brood from the colony the mites are removed too. Additionally, when the mites are on the bees, particularly when there is no brood in the colony, the efficiency of the treatments improves significantly.

METHODS FOR BROOD INTERRUPTION

This handbook describes three methods for brood interruption in summer by applying which the level of mite infestation can successfully be controlled in the honey bee colonies:

1. Brood removal
2. Queen caging
3. Trapping comb

The application of the methods "Brood removal" and "Trapping comb" on productive honey bee colonies completely rules out the use of chemicals, while the method "Queen caging", which is a combination of biotechnical and chemical treatment, uses oxalic acid. Due to this, these methods are an excellent alternative to synthetic chemicals for mite control.

An additional advantage of the methods for brood interruption is the possibility to adapt them to the beekeeping conditions. Thus, depending on beekeepers' individual needs or limitations, the methods could be integrated into various beekeeping practices.

Namely, the application of the methods could be used to replace dark, irregularly built or deformed combs, to replace queens if necessary, for production of new colonies, etc. Generally speaking, the methods for brood interruption in the summer period can be successfully applied in the majority of modern types of hives.

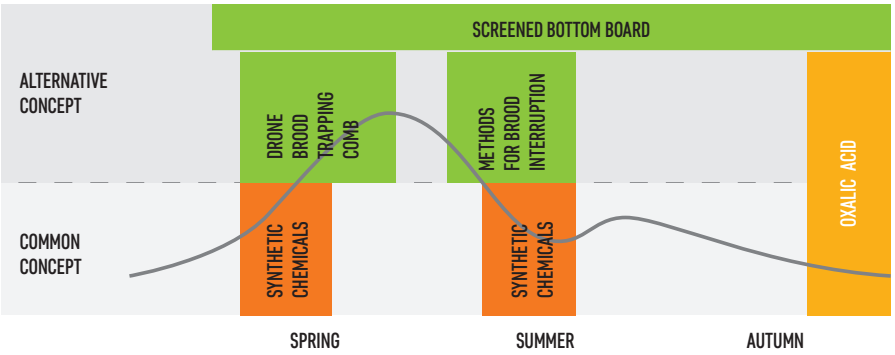
The significant difference, and at the same time advantage of the methods for brood interruption, compared to treatments with synthetic chemicals, is the period of application in relation to honey harvest. With regard to the risk of residues, treatments with synthetic chemicals can only be applied after honey extraction, while there is no such limitation for the brood interruption methods, which allow flexibility in the period of application. Only in the method of "Queen caging" the honey should be taken out of the hives before the oxalic acid is applied. However, the decision when to apply the methods depends mostly on the conditions and the concept of beekeeping of individual beekeepers.

A common timing for application of the methods for brood interruption in summer is towards the end of foraging. With the accessibility of nectar and pollen, the colonies where the methods are applied have the opportunity for renewal, i.e. preparation for the forthcoming period in a brief

amount of time. This is a crucial aspect since the application of these methods is a major change and interruption in the development of the colony.

Among the methods for brood interruption, there are differences in the complexity of their application, which, on the other hand, means limited applicability for different beekeeper profiles. The "Queen caging" method is probably the most acceptable for the majority of beekeepers, while the "Brood removal" and "Trapping comb" are more acceptable for hobby beekeepers or those with smaller production capacity. Another difference in the application is the handling (manipulation) of the queen, so that in the "Brood removal" method there is no need to find it and close it in a cage or isolator. In any case, regardless of the beekeeper's profile and the intensity of production, the bio-technical methods for brood interruption in summer can be applied and incorporated in the concept of organic beekeeping.

With a proper application of the methods for brood interruption and efficient summer treatment, the control of mites in the colonies can be achieved with only two treatments per year. Thus, in addition to the summer treatment with some of these methods, the colonies can be additionally treated with oxalic acid in the period when there is no brood, i.e. late autumn or early winter (November - December). In this way, apart from the exclusion of synthetic chemicals during the year, the number of treatments is also reduced (Figure 7). This is a significant difference because some beekeepers commonly treat honey bee colonies more than twice per year (spring, the summer – after the honey is harvested, and winter) when synthetic chemicals are mostly used.



7 Comparison of common and alternative "nature-like" concept for mite control.

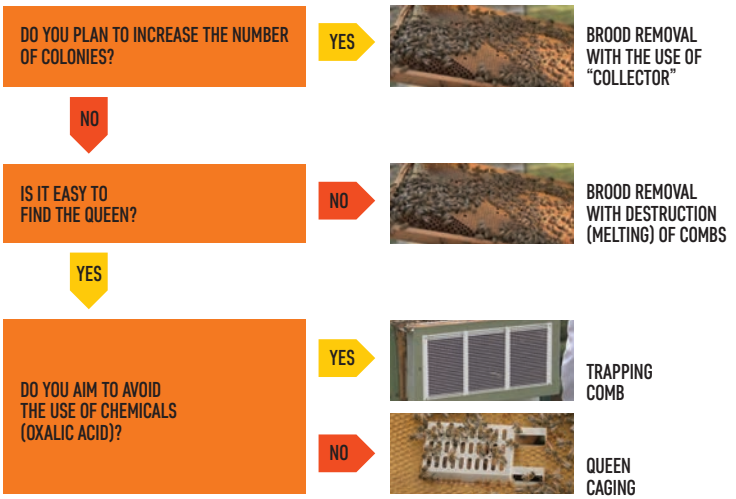
The success of mite control throughout the year also depends on the application of other bio-technical methods. That is why, in addition to the methods for brood interruption in summer it is advisable to use screened bottom boards during the whole year, while the use of drone brood trapping combs is particularly important in spring.

Additionally, keeping selected genotypes of honey bees, particularly those that are the product of specialized programmes for selection for resistance to mites, is an approach that fits perfectly in

the concept of year-round mite control with the application of the methods for brood interruption in summer.

REARING NATIVE AND LOCAL HONEY BEE POPULATIONS IS A STRATEGICALLY JUSTIFIED APPROACH THAT SHOULD BE THE FOUNDATION OF MODERN BEEKEEPING.

When choosing the method for brood interruption in summer, beekeepers should take into account the above-mentioned aspects, and at the same time, assess how the chosen method will be incorporated in their beekeeping practices. In order to help beekeepers in the decision-making process, the Bee Institute in Kirchhain, Germany, developed a schematic for the selection of the method for brood interruption summer (Figure 8).



8 Schematic for selection of method for brood interruption (Bee institute in Kirchhain, Germany, 2017).

The particular recommendations for the application of each of the methods are given in the description of the methods, while the general recommendations, as well as the recommendations for monitoring the level of infestation, are given in a separate chapter "General Recommendations for Monitoring and Application of the Methods".

1

BROOD REMOVAL

The reproductive phase of *V. destructor* takes place in the sealed worker or drone brood, which is an opportunity to "capture" it and together with the frames with combs with sealed brood, remove it from the colony. In fact, first, open and sealed brood are removed, with the exception of one "trapping" comb with open brood with eggs and larvae at different stages. Nine days later, the "trapping" comb, now with a mostly sealed brood and "captured" mites, is removed from the colony. In this way, the first removal of sealed brood eliminates many of the mites from the colony. However, with the removal of the open brood, the remaining mites on adult bees can only access the brood in the "trapping" comb. Finally, with the removal (after 9 days) and melting of the "trapping" comb, part of the remaining mites in the colony is eliminated. The frames with brood from several productive honey bee colonies, on which the method is applied, are collected in a so-called "collector", which should be later treated. With this method, by removing brood from full-sized and productive colonies, no chemicals are used in the productive colonies.

The application of the method is a radical interruption in the colony development, so it is necessary to carefully assess the conditions in the forthcoming period, primarily the availability of nectar and pollen, so that the colonies have the opportunity to renew themselves in a short period of time. Because of this, this method is particularly suitable for colonies kept in regions with extended brood rearing in the season.

Having in mind that this method requires additional equipment (bottom boards, boxes, frames with built combs or comb base, etc.), and is also demanding in terms of engagement, it is more attractive for hobby beekeepers and those with a smaller number of colonies. On the other hand, the method can be combined with the production of new colonies, which is an additional aspect, interesting for all types of beekeepers.

PREPARATION AND EQUIPMENT

- Additional bottom boards, hive boxes, inner covers, outer covers, frames with combs and comb foundations.
- Oxalic acid for treatment of the "collector" and equipment for its application.

STEP BY STEP

... to watch the video for method application

STEP ONE

9 Removal of combs with brood and food and transfer to "collector".

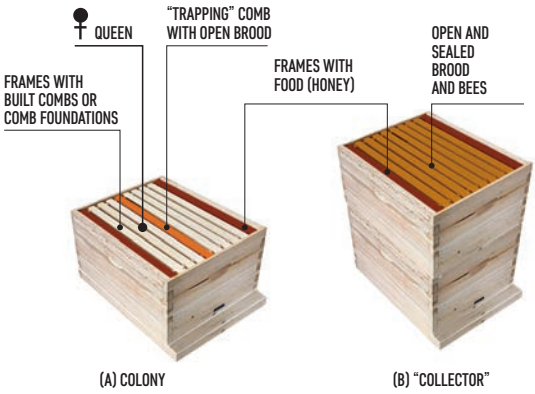
On the first day of the method application, the frames with combs with open and sealed brood are removed from the hive and transferred to the "collector" (Figure 9). Attention should be paid so that the queen remains in the colony, where it continues laying eggs without interruption.

On each side of the frames that are transferred 200 to 300 worker bees are left (Figure 10) so that in the first several days the minimum necessary conditions and brood care are provided in the "collector".

10 Comb with brood and food and around 200 worker bees.

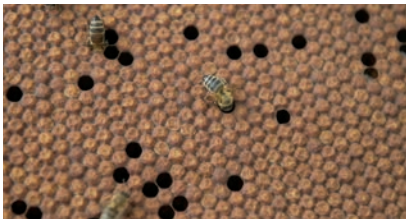
In the "collector", 1 to 2 frames with honey (food) should be added for each box with brood combs, and then it would be best to move the "collector" to a new location.

In the middle of the original colony only one frame is left, with an open brood with eggs and larvae at different ages, which would serve as the "trapping" comb (Figure 11). Frames with built combs are introduced in the vacated spots in the hive, and in case there are no such combs, frames with comb foundations are introduced.



11 (A) A colony after brood removal and (B) "collector" of frames with brood and food from several colonies.

STEP TWO



12 Sealed brood on the "trapping" comb.

Nine days after the brood removal, the "trapping" comb, now with a mostly sealed brood (Figure 12) and "trapped" mites (Figure 13), is removed from the hive and replaced with a frame with built comb or comb foundation.

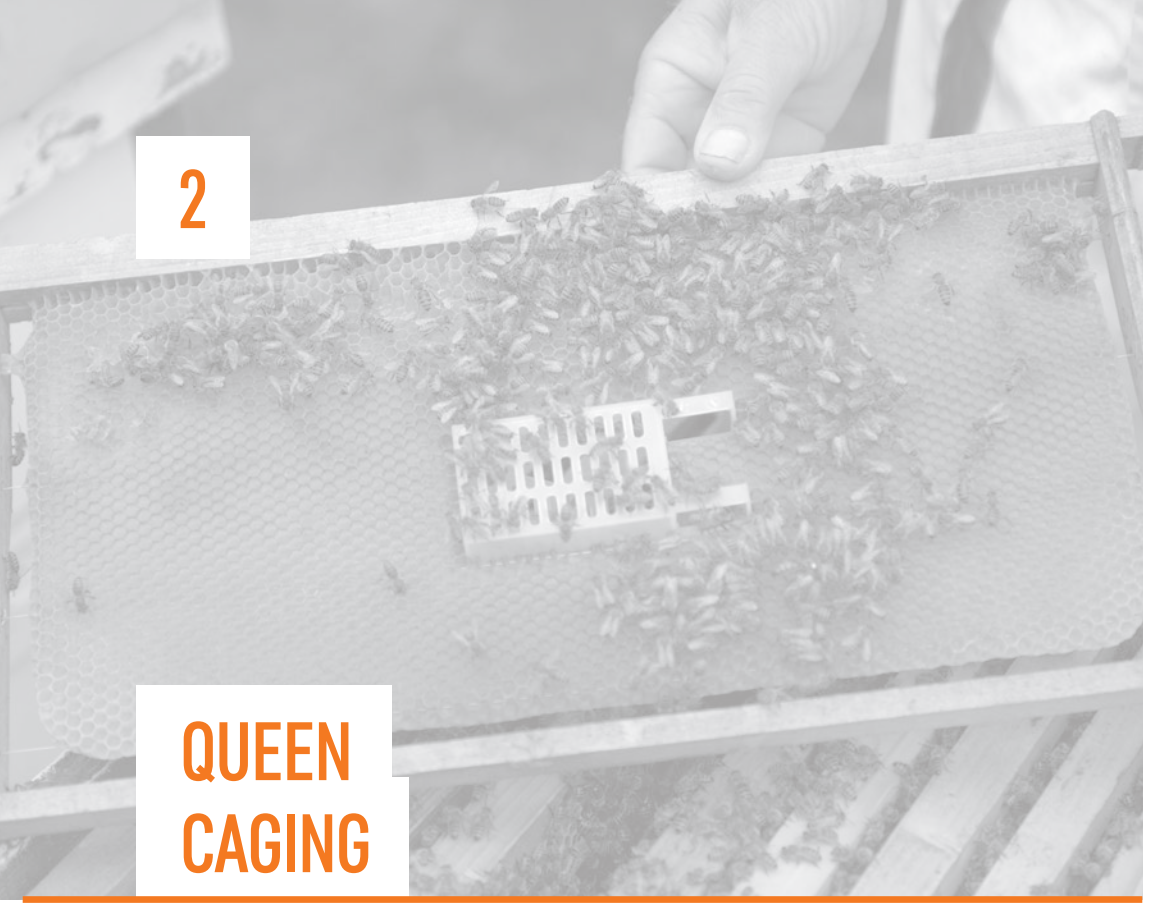
13 *V. destructor* in a sealed brood.

STEP THREE

Three to four weeks after it's established, the "collector" is treated with oxalic acid. At the same time, it is checked for the presence of a new queen, the frames with dark, irregular or deformed combs are removed, as well as the unnecessary boxes.

RECOMMENDATIONS

- Period of application: 1 to 2 weeks before the foraging ends or immediately after the honey harvest.
- For the efficient and quick application of the method, it would be best to have the support of an additional, experienced person.
- If the "trapping" comb contains drone brood, the method can be expected to be more efficient.
- The "trapping" comb should not be reused, so it would be best to melt it and use the wax or destroy the comb with the brood.
- If in step one, in addition to the open brood there is a smaller area with a sealed brood on the "trapping" comb, it should be destroyed by scratching.
- The small areas with brood, on a comb with a significant quantity of food, could be destroyed with scratching or cut off with a scalpel. In this way, the frame with food can remain in the productive bee colony.
- Due to the complexity of the method, it is recommended that in the first years, the experience should be gained and the method tested on a smaller number of colonies.
- With the application of this method, it is possible for new colonies to be established late in the season. However, the management of these colonies as well as the treatment for mite control, require beekeeping experience and previous practise with the method.
- There is a modified model of this method, when after the brood removal, oxalic acid is immediately used, without the use of the "trapping" comb. At the same time, the removed brood can be destroyed.



2

QUEEN CAGING

The method, which is a combination of biotechnical and chemical treatment, is based on the fact that mites, when there is an interruption and absence of brood in the colony, which is achieved with confining the queen for a period of 25 days, are located on adult bees. In such a situation, oxalic acid is applied to the colony with the method of trickling (or another method of application), which achieves an effective summer treatment for *Varroa* control.

Many beekeepers are familiar with oxalic acid as a chemical used for autumn/winter treatment for mite control when there is no brood in the colony, or it is present only in minimum areas. In this way, in the annual mite control concept, beekeepers use a chemical with which they have the experience, which significantly reduces the risk for wrong application or the beekeeper being injured during the activity. However, the greatest benefit for beekeepers, as well as consumers, is that the application of this method completely rules out the use of synthetic chemicals. Oxalic acid is the recommended chemical in organic beekeeping, and research shows that the efficacy is over 90% in brood-less colonies or less than 60% in brood-right colonies.

This method is acceptable to almost all beekeeper profiles and can be applied under different intensity of beekeeping production.

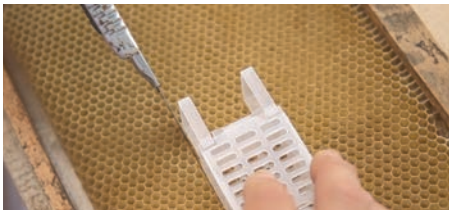
PREPARATION AND EQUIPMENT

- An especially designed queen cage (Figure 14), with sides made of a queen excluder (Hanemann screen), which only the worker bees can traverse and be in constant contact with the queen.

14 Cage for confining the queen.

- Knife, scalpel or another kind of blade.

- During the first use, using a sharp blade to cut the wax comb, the cage is inserted into a frame with a built comb (Figure 15 and 16).



15 Attaching the cage.

16 Frame with the queen cage attached to a comb.

- Oxalic acid and equipment for handling and applying it by trickling method (Figure 17).

17 Equipment for handling and applying oxalic acid.

STEP BY STEP

... to watch the video for method application

STEP ONE

18 Introducing the queen in the cage.

The queen is located in the colony and carefully introduced in the cage (Figure 18).

19 Placing the frame with the cage in the colony's central position.

Immediately afterwards, the frame with the cage is placed in a central position in the colony (Figure 19) and is marked.

STEP TWO

20 Releasing the queen from the cage after 25 days.

On day 25 of the caging of the queen, the cage is opened, and the queen is released (Figure 20), and with the method of trickling oxalic acid is applied directly over the bees in the space between the frames (Figure 21).

21 Applying oxalic acid with the method of trickling.

RECOMMENDATIONS

- Period of application: 2 to 3 weeks before the end of foraging or immediately after the honey harvest. In any case, oxalic acid should be applied after the honey is collected from the hive.

- It is recommended for the frames with attached cages to be prepared in advance so that the application of the method in the apiary is straightforward and faster.

- If the beekeeper has the opportunity, 7 to 9 days after caging the queen, it is recommended to check for the possible presence of emergency queen cells, which should be destroyed if present.

- The recommended concentration for summer treatment with trickling oxalic acid is 4.2% (5 ml per occupied comb with bees) (Büchler et al., 2019).

- It is best to apply oxalic acid in the late afternoon when the foraging bees are already in the hive.

- Transportation cages for queens should not be used as an alternative.

- The old or unproductive queen can be replaced with a new one.

- Vigilance when handling oxalic acid, respecting the recommendations for proper application and obligatory use of protective gear!

3

TRAPPING COMB[4]

The “Trapping comb” method partially incorporates elements from the two previously described methods. The isolation of the queen, on a comb that is introduced in the “isolator” (Figure 22), significantly reduces the area of brood that is available for mite reproduction.

22 "Isolator" with sides made of queen excluder.

In the isolated frame, the queen freely lays eggs, which a few days later are the only possibility, i.e. available brood (“trap”) in the colony for mite reproduction. After the brood is sealed, the frame is removed, thus eliminating the “captured” mites. The procedure, with new combs in the “isolator”, is successively repeated two more times, after which the queen is freed, and the colony returns to its natural state.

Unlike other methods for brood interruption, with this method, the situation of the colony is not disrupted so drastically, while at the same time, the queen can lay eggs without interruption. However, the application of this method demands a more intense engagement and frequent visits to the apiary by the beekeeper. Because of this, this method is particularly attractive for hobby beekeepers and beekeepers with a small number of colonies.

PREPARATION AND EQUIPMENT

- A “isolator” with sides made of queen excluder (Hanemann screen) which only the worker bees can traverse.
- Frames with built combs.

[4] The method was not tested in the project “Alternative approach for *Varroa destructor* control in the summer period”

STEP BY STEP

... to watch the video for method application

STEP ONE

23 Introducing the queen on frame 1 in the "isolator".

Frame 1, with an empty drawn comb where brood was reared, is introduced in the "isolator", together with the queen (Figure 23).

24 "Isolator" with frame 1, placed in a central position.

The "isolator" is then placed centrally in the colony (Figure 24).

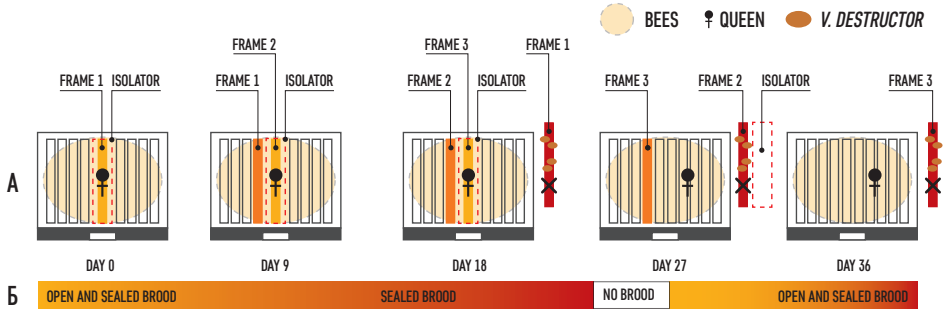
STEP TWO

25 Frame 1 to the "isolator" containing frame 2 and the queen.

Nine days later frame 1 is removed from the "isolator" and is placed immediately next to it. Frame 2 is introduced in its place (Figure 25) when the queen remains in the "isolator". Also, it is recommended to check the frames with brood for the possible presence of emergency queen cells, which should be destroyed if present.

STEP THREE

Nine days later, or the 18th day from step one, frame 1, now with a sealed brood and "trapped" mites, is removed from the hive. Frame 2 is removed from the "isolator" and placed immediately next to it. Frame 3 is introduced in its place in the "isolator" (Figure 26).



26 (A) Step by step procedure during the application of the method Trapping comb and (B) the state of the remaining brood in the colony, not considering the "trapping" combs.

STEP FOUR

Nine days later, or 27 days after step one, frame 3 is removed from the "isolator" and left in the hive, while the queen is released. At the same time, frame 2 and the "isolator" are removed from the hive (Figure 26).

STEP FIVE

Nine days later, or on day 36 after step one, frame 3 is removed from the hive and is checked whether the queen lays eggs normally and regularly (Figure 26).

RECOMMENDATIONS

- Period of application: 2 to 4 weeks before the end of foraging or immediately after the honey harvest.
- It is recommended that the frames with empty combs inserted in the "isolator" have been previously used for brood rearing so the queen will readily lay eggs.
- The combs that were put in the "isolator" are not reused, so the combs should be melted or destroyed.
- Due to the "isolator's" width, it is necessary to make room by removing one or two frames, with preferably dark, irregularly built or deformed combs.
- There is also a so-called weekend version of the method, with 4 "trapping" combs at a seven-day interval.

GENERAL RECOMMENDATIONS FOR MONITORING AND APPLICATION OF METHODS

MONITORING

One of the postulates of good beekeeping practice is regular and systematic monitoring, i.e. checking the health status of the honey bee colonies, primarily the level of infestation with the *V. destructor*. The ideal approach would be to monitor the infestation in all colonies.

Apiary size (Number of colonies)	Representative sample size (number of colonies)
≤ 10	All
≤ 20	6-10*
> 20	At least 8*

However, having in mind that the application of the monitoring methods requires the beekeeper to invest time and effort, it would be realistic to expect that monitoring can be done only on a part (representative sample size) of colonies (Table 1).

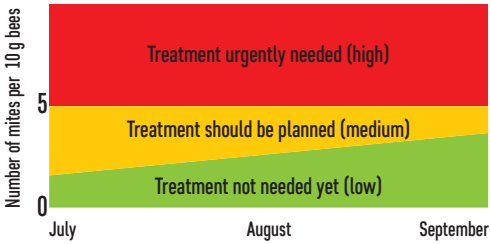
T1 Suggested number of colonies to be sampled depending on the total number of colonies in the apiary (according to Véto-pharma).

*The sample should include colonies of different strength.

27 "Powdered sugar" method.

There are several monitoring methods, but the most widespread among beekeepers is the "powdered sugar" method[5], which determines the number of mites on 50 g worker bees, and is expressed as a number of mites per 10 g worker bees (≈ 100 worker bees) which can be seen as an approximate percentage of infestation (Figure 27).

This method is particularly relevant for the period after the summer treatment for mite control (July to September), and in this case, after the application of methods for brood interruption. The goal is to determine the methods' efficacy, i.e. the level of infestation in the colonies and consequently, if necessary, take additional actions.



G1 Threshold values of infestation of the colonies in summer and recommended actions.

Graph 1, based on the level of infestation in colonies (mites per 10 g worker bees), gives recommendations for actions so that beekeepers can control mite infestation during the summer.

In case of a medium, and particularly in a high level of infestation, additional urgent interventions are necessary to control the mite population. Which type of treatment the beekeeper will choose depends on the infestation level and the period for treatment.

[5] http://bit.ly/seker-vo-prav - Bee Institute in Kirchhain, Germany (SMARTBEES - project).

RECOMMENDATIONS FOR PROPER APPLICATION OF THE METHODS FOR BROOD INTERRUPTION

In addition to the specific and particular recommendations given in the description of the methods, there are also common or general recommendations for a more successful application of the methods:

- It is recommended for the methods to be applied near the end of the main foraging or at the time of so-called quiet nectar flow, but not late in the season.
- In case of a period without nectar flow (or a long rainy period), it is necessary to feed the colonies with sugar syrup.
- Reduce the risk of robbing between colonies with a) good planning of activities and efficient work, b) application during foraging or c) in the period of the day with low activity of foraging bees (e.g. in the afternoon).
- The equipment and the tools should be prepared on time to ensure efficient work without interruptions.
- For the easier application of the procedures, the queens should be marked.
- The methods could be modified or combined so that they can be better integrated into the beekeeping practices of the individual beekeepers.
- Before they are introduced in regular practice, the methods should be tested and applied on a smaller number of colonies, so that the beekeeper gains the necessary experience.
- Use of selected native or local genotypes of honey bees, resistant to the *Varroa* mite.
- The methods are an excellent opportunity to replace the dark, irregularly built or deformed combs.

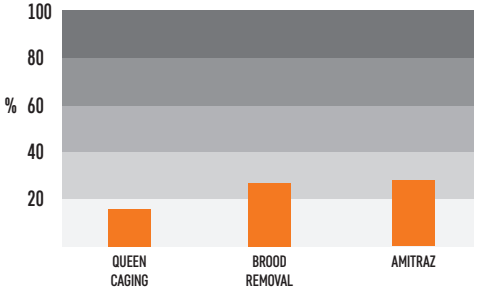
EXPERIENCE WITH METHOD APPLICATION

Practical experience with colonies in different parts of Europe confirmed that the application of the methods for brood interruption is a realistic and successful approach for control of *V. destructor* in summer.

The results and information presented in this chapter derive from the research conducted as part of the two-year (2017-2019) project "Alternative approach for *Varroa destructor* control in the summer period". Nine beekeepers from the Bregalnica region, with a total of 90 honey bee colonies, took part in the research when the methods "Queen caging" and "Brood removal" were applied in summer and compared with the traditionally used method of applying amitraz-based products. Besides, there are also results from other similar research and applicative projects in North Macedonia, Germany and Italy. It should be mentioned that during the implementation of the project in the Bregalnica region, the colonies were treated only in summer those two years, which is significantly different from the common approach of treating the colonies two or three times a year. Also, most of the beekeepers who participated in the project were beginners, with two years of beekeeping experience.

INCIDENTS WITH QUEENS AND COLONIES[6]

The beekeepers' concern in terms of risks for colony losses or queens being lost or replaced during the application of the methods seems to be unfounded.



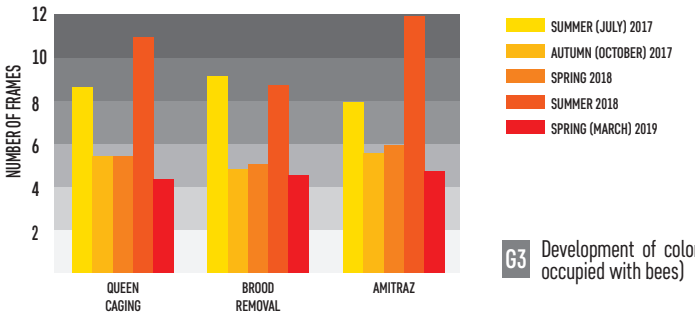
G2 Total percentage of incidents with queens and colonies in a two-year period.

Namely, the percentage of incidents with queens and colonies in the two-year period, when the methods of "Queen caging" and "Brood removal" were applied, was lower than the percentage among the colonies treated with amitraz with fumigation (Graph 2). Contrary to expectations, the percentage of incidents was lowest with the method "Queen caging", which shows that the caging of the queen for 25 days does not pose a significant risk for the queen and the colony.

Interestingly, the survey carried out among the beekeepers at the beginning of the research showed that the majority were concerned about the risk of loss or negative consequences for the queen and the colonies during the application of the "Queen caging" method. However, the percentage of incidents for all groups in the first year was lower than the second year, which might be the result of the queens' age. Experiences are similar in other previous research in North Macedonia, Germany and other European countries.

DEVELOPMENT OF COLONIES

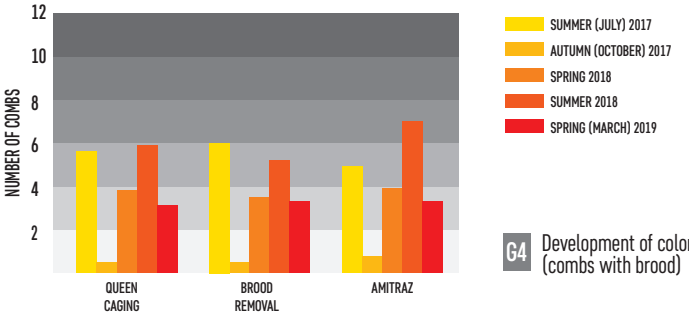
In terms of development of the colonies, measured by the number of frames occupied by bees and the number of combs with brood, the differences between the groups of colonies, i.e. the methods, are even smaller (graphs 3 and 4). This should be underlined because in the colonies from the groups "Queen caging" and "Brood removal", in the period immediately after the honey was harvested, the queens were closed in cages, or the brood was removed.



G3 Development of colonies (frames occupied with bees)

[6] Lost or supersedured queens and colony loss.

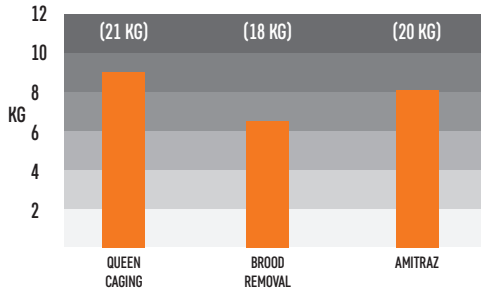
This leads us to the conclusion that the colonies in these groups, after the method application, managed to compensate for the brood interruption and get ready for winter. Still, compared to other methods, it seems that "Brood removal" has a greater impact on development, because these colonies had the lowest number of combs with bees and brood.



G4 Development of colonies (combs with brood)

The results were similar with the research carried out in the Bee Institute in Kirchhain, Germany, where the colonies that underwent "Queen caging" and "Brood removal" in summer (July), were more developed the following spring, i.e. had more bees and a brood than the colonies treated with formic acid.

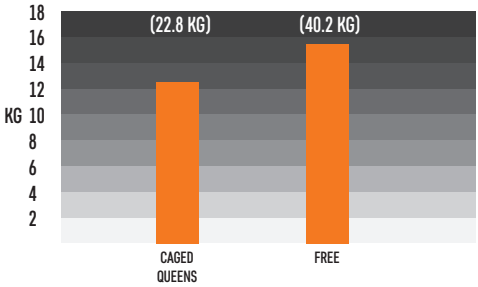
HONEY PRODUCTION



The differences between groups in terms of the development of colonies also affected the production of honey in 2018. Thus, most honey on average was made by colonies where the "Queen caging" method was applied the previous year and least by colonies with the "Brood removal" method (Graph 5).

G5 Average honey production in 2018.
The maximum values of harvested honey are given in brackets

In any case, it should be emphasized that the honey and other products from the colonies where the methods were applied are expected to be better recognized and valued on the market because no synthetic chemicals were used for mite control.



G6 Honey production in 2018 by colonies with caged queens and colonies with queens free in the brood chamber. The maximum values of produced honey are given in brackets

Additional tests were done in three different locations in the Bregalnica region in an attempt to respond to the frequently asked question: "*Will the application of the "Queen caging" method in the period of most intensive foraging period, lead to increased honey production?*" The colonies whose queens were free in the brood chamber produced on average 3 kg more honey compared to colonies whose queen were caged during the most intensive foraging period (Graph 6).

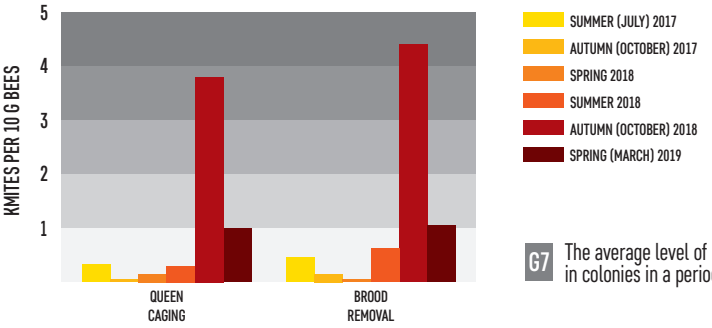
However, it is necessary to mention that these results are from one year. The additional research carried out in several regions in Europe in 2019 gave contradictory results, which show that this important issue should be additionally studied.

Research in Germany showed that when the method "Brood removal" was applied, the average honey production was 27.3 kg per colony when the method was applied 2 weeks before the last honey harvest (mid-July). This is significantly more than the honey produced when the method is applied in May (7.1 kg) and June (16.4 kg).

INFESTATION WITH V. DESTRUCTOR AND INCIDENTS WITH OTHER PATHOGENS

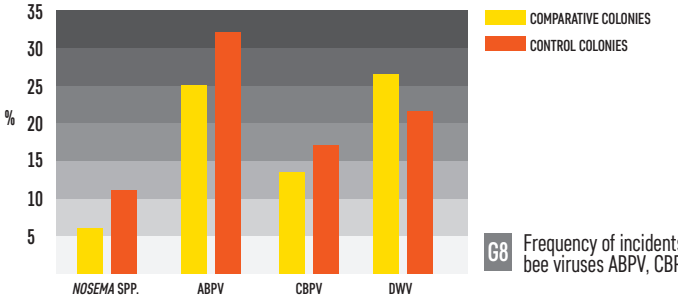
Research results so far show that the methods for brood interruption in summer have big potential for reducing the level of mite infestation in the colonies. The average level of *V. destructor* infestation in the colonies with "Queen caging" and "Brood removal", during the entire research period, was below the critical level of 5 mites per 10 g bees (Graph 7). As expected, because the autumn-winter treatment was not applied, the average infestation was higher in the second season of the research, yet it was still below the critical level.

These results are encouraging, having in mind that the colonies were treated only in the summer, i.e. two treatments in two years. Unlike the regular beekeeping practice in North Macedonia, this is a significantly smaller number of treatments for mite control annually. With an additional autumn-winter treatment of the colonies, the level of mite infestation can be successfully controlled during the whole year, which is a precondition for keeping vital and productive honey bee colonies, which are expected to have top performances and production results the following year.



G7 The average level of mite infestation in colonies in a period of two years.

In terms of the frequency of incidents with *Nosema* spp. and bee viruses[7] (ABPV, CBPV and DWV), in research carried out at the Bee Institute in Kirchhain, Germany, there were no significant differences between the colonies from the "Brood removal" group and the comparative group, where the common method with formic acid was applied (Graph 8). In any case, in the colonies where the method "Brood removal" was applied there was a lower frequency of ABPV and CBPV, as well as *Nosema* spp., which means that in addition to the impact on mite population, this method can also influence and improve the overall health of the honey bee colonies.



G8 Frequency of incidents with *Nosema spp.* and bee viruses ABPV, CBPV and DWV (Büchler, 2009).

[7] ABPV – Acute bee paralysis virus; CBPV- Chronic bee paralysis virus; DWV – Deformed wing virus.

APPLICABILITY, TIME AND RESOURCES NECESSARY FOR METHOD APPLICATION

In the experience of beekeepers who participated in testing the methods in the country and abroad, as well as the authors' experience, there are differences in the applicability of the three above-mentioned methods for mite control (Table 2).

	Support by an additional person	Time (minutes)*	Steps	Use of chemicals	Complexity	Beekeper profile
Brood removal	YES	25-35	3	NO	Medium to high	Hobby and small
Queen caging	NO	15-20	2	YES Oxalic acid	Moderate	All
Trapping comb	NO	30-40	5	NO	Medium to high	Hobby and small

T2 Assessment of method applicability.

*Including all relevant procedures for method application per colony.

"Queen caging" is the most acceptable method for beekeepers in different climate conditions, the intensity of beekeeping (hobby, additional activity or commercial) as well as for the beekeepers without long experience. It should also be mentioned that this method is a combination of biotechnical and chemical treatment with oxalic acid, which is the recommended substance for *Varroa* control in organic beekeeping. Also, the methods of "Brood removal" and "Trapping comb", where no chemicals are used for controlling the mite, are particularly attractive and applicable in organic beekeeping. In any case, these three methods are an alternative to synthetic chemicals for mite control.

By raising customers' awareness of consuming and using safe and good-quality honey bee products, there is a need to introduce a new and contemporary concept of beekeeping. This concept implies the application of bio-technical methods for mite control, non-use of synthetic chemicals, systematic monitoring of colonies' health in order to optimize the number of treatments, use of queens from select genotypes from the native population (*A. m. macedonica*), as well as continuous education and training.

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