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ORIGINAL RESEARCH ARTICLE

Summer brood interruption as integrated management strategy for effective Varroa control in Europe

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Most Varroa induced colony losses occur during the autumn or winter season as a consequence of an elevated Varroa infestation level and an insufficient health status of the adult bees. Even with an initial low Varroa infestation in early spring, critical mite and virus infection levels can be reached before winter if colonies continuously rear brood throughout the whole season. To overcome this challenge, beekeepers can artificially interrupt brood production by suitable management procedures, depending on their type of beekeeping operation. To assess their efficacy, associated workload, and impact on colony development, different methods for brood interruption (queen caging with the combination of oxalic acid treatment, total brood removal, trapping comb technique) were tested during two seasons in 11 locations on 370 colonies in 10 European countries. A protocol was developed to standardize the methods' application across different environmental conditions. The efficacy of queen caging depended on the mode of oxalic acid application and ranged from 48.16% to 89.57% mite removal. The highest efficacies were achieved with trickling a 4.2% solution (89.57%) and with the sublimation of 2 g of oxalic acid (average of 88.25%) in the broodless period. The efficacy of the purely biotechnical, chemical-free trapping comb and brood removal methods did not differ significantly from the queen caging groups. We conclude that a proper application of one of the described brood interruption methods can significantly contribute to an efficient Varroa control and to the production of honey bee products meeting the highest quality and food-safety standards.

Keywords: Varroa mite; biotechnical control; brood interruption; colony losses

Introduction

Annual colony losses of about 10 – 30% of the total honey bee (*Apis mellifera*) population commonly occur in many European countries (Brodschneider et al., 2016; 2019; Chauzat et al., 2014). Most losses happen during the winter season and are closely correlated with high *Varroa destructor* infestation levels of the hives during the period of winter bee rearing in the previous autumn (Hatjina et al., 2014; Meixner et al., 2014). This can be explained by the negative effect of Varroa parasitism on the development of the fat bodies and vitellogenin content of winter bees (Amdam et al., 2004; Ramsey et al., 2019), directly influencing their life expectancy. A steep increase of winter mortality was noted for colonies with critical infestation levels (Genersch et al., 2010) caused by five to six months of continuous brood rearing (Martin, 2002).

Under natural conditions, seasonal brood interruption usually occurs through the process of swarming and results in reduced Varroa levels in overwintering colonies (Fries et al., 2003; Ratti et al., 2016; Szabo, 2008), even without the application of drugs. However, in modern beekeeping natural swarming is usually prevented, in order to keep the colonies as strong as possible throughout the season, and to maximize the honey yield.

To avoid serious colony damage due to an excessive Varroa infestation level, many beekeepers have to treat their colonies during summer, stopping the honey

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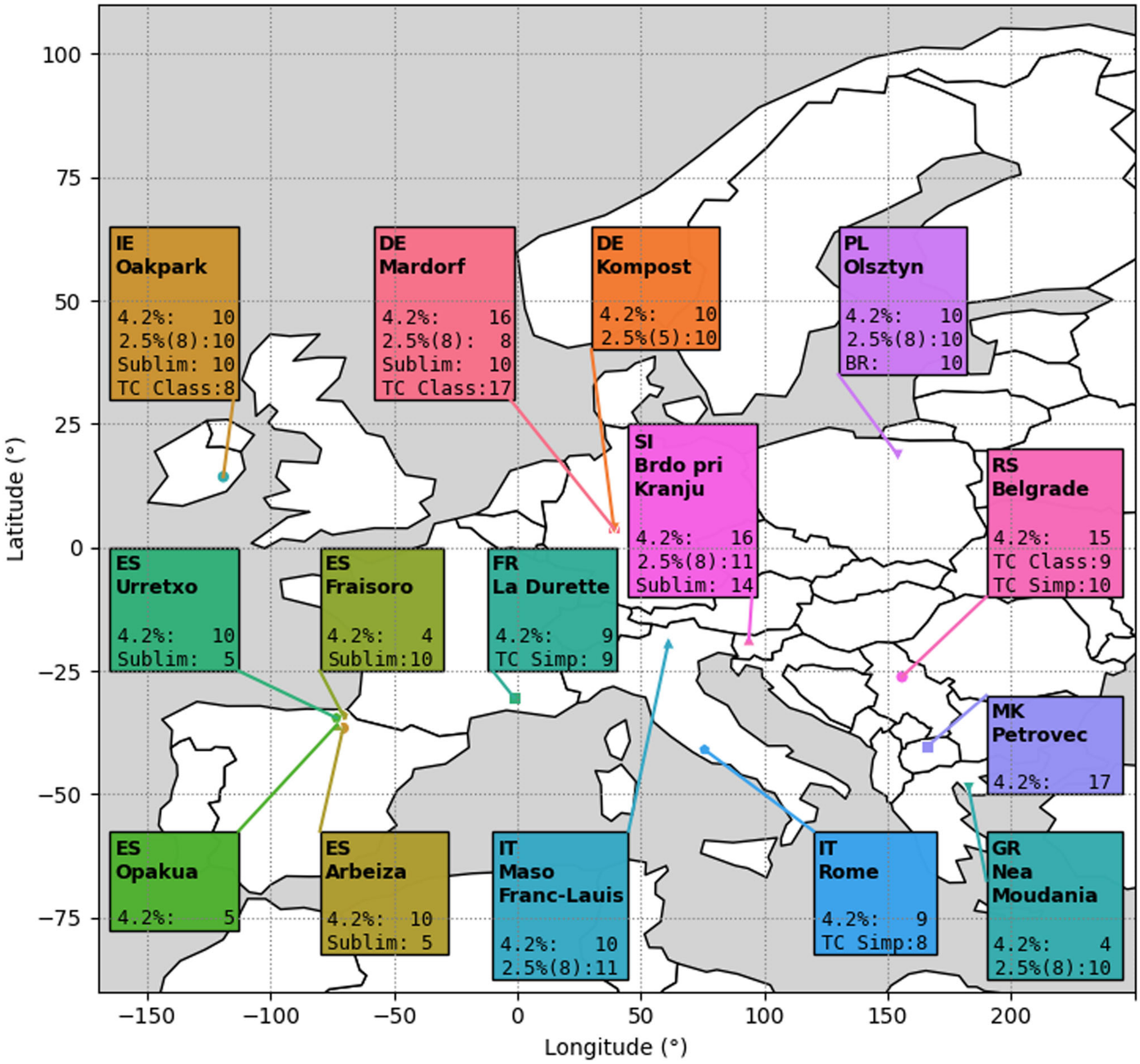

Figure 1. Map of participating apiaries. All methods used in the apiary are presented together with the colony count per method. 4.2%: queen caging with trickling of 5 ml 4.2% oxalic acid; 2.5% (5): queen caging with trickling of 5 ml 2.5% oxalic acid; 2.5% (8): queen caging with trickling of 8 ml of 2.5% oxalic acid; Sublim: sublimation of 2 g oxalic acid; TC(Class/Simp): trapping comb (classic/simplified); BR: Brood removal. 4.2% was applied as a standard treatment in all apiaries.

harvest (Martel et al., 2007; Wallner, 1999) in observation of the withdrawal time, as most registered therapeutics may result in residues in hive products. Moreover, recurrent problems of resistance were observed after intensive acaricide treatments (Milani, 1999; Mozes-Koch et al., 2000; Sammataro et al., 2005; Thompson et al., 2002) Finally, most registered drugs do not affect Varroa mites inside the brood cells, and their efficacy is not sufficient in colonies that reared brood during the whole season (Gregorc et al., 2016).

Varroa mites infesting brood can only be affected by evaporating agents like formic acid (Fries, 1991; Rosenkranz et al., 2010). However, evaporation rates depend on the temperature and humidity inside and outside the hive and therefore affect the success of treatment (Calderone, 1999; Underwood & Currie, 2003). Under suboptimal environmental conditions, the beekeepers risk either to harm their bees (Bolli et al., 1993; Satta et al., 2005) or to achieve an insufficient efficacy against the mites (Calderone, 1999; Eguaras et al., 2003).

Reliable and high treatment effects can more easily be achieved in broodless colonies. An induced brood interruption can, therefore, be a useful tool to improve treatment efficacy and to limit periods of continuous mite population growth. Furthermore, if beekeepers generally want to avoid the use of any chemicals or have to treat during long honey flow seasons, they can

Table 1. Overview of the tested methods.

Method	Abbreviation	Mode of application	Product
Queen caging	4.2%*	4.2%oxalic acid, trickling, 5 ml per occupied comb	Apibioxal®
	2.5% (5)	2.5%oxalic acid, trickling, 5 ml per occupied comb	Oxuvar®
	2.5% (8)	2.5% oxalic acid, trickling, 8 ml per occupied comb	Oxuvar®
	Sublimation	2 g oxalic acid dihydrate crystals, sublimation	Varrox®
Trapping comb	TC Classic	Queen caged on a single comb over a period of 9 days, and 3 repetitions (3 × 9 days)	
	TC Simplified	Queen caged on a single comb over a period of 20 days, oxalic acid 4.2 % trickling at day 25	
Total brood removal	Brood removal	Removal of all brood combs and use of a single comb with open brood over a period of 9 days and subsequent removal	

*Standard method applied in all locations.

combine controlled brood interruption with the use of trapping combs (Maul et al., 1988). To do so, some open brood is left in otherwise broodless colonies, which will attract most of the mites that are on the adult bees. The brood can afterwards be removed from the hive together with the trapped mites.

In this large-scale study, we compared the effect of different methods of controlled brood interruption in combination with either chemical or exclusively biotechnical mite control with regard to their efficacy, their effect on colony development, and their practicability and workload. In all involved apiaries, the caging of the queen for 25 days in combination with a 4.2% oxalic acid trickling treatment at the end of the caging period was used as a standard treatment (Nanetti et al., 2011). With regard to different registrations between the countries, caging was also combined with different concentrations, dosages, and application modes of oxalic acid. Furthermore, in some apiaries we tested the use of full-size brood frame cages to limit brood production to single trapping combs and total brood removal, which can be performed without having to search for the queen and handle it repeatedly.

Materials and methods

The study was conducted during the seasons 2016–2017 and 2017–2018 in 10 European countries and 15 locations (Figure 1) with a total of 370 colonies representing different European honey bee populations. In order to achieve comparable data, the experimental design required for each participant an apiary of at least 20 full size, naturally infested colonies, and the set-up of two or several groups of at least ten colonies each. Data on colony strength and mite infestation level of bees had to be measured at the beginning of the experiment in order to distribute the colonies into homogeneous groups.

The efficacy of different methods against Varroa was compared to the standard method of queen caging with the application of 5 ml 4.2% oxalic acid per occupied comb (Table 1 & Figure 2). A standardized testing protocol with detailed information about the methods and their application was developed and published on the COLOSS website (A copy is available in supplemental online material).

Briefly, in this method, the queen was caged for 25 days in a specific cage of 5.0 * 7.5 * 2.5 cm size (http://www.apimobru.com/) that was fixed in a comb frame. At the time of queen release (day 25), oxalic acid was trickled (Table 1).

The trapping comb method (TC) was implemented in two different ways: a classic one and a simplified one. In TC Classic, the queen was caged on an empty comb to produce limited areas of open brood. After 9 days, it was moved to an empty comb, while the first comb remained in the brood chamber as a trapping comb for mites. On day 18, the original and meanwhile sealed trapping comb was removed from the hive, and the second trapping comb remained in its position while the queen was caged on a third comb. The queen was finally released on day 27, when the second trapping comb was removed from the hive. The third trapping comb remained in the broodnest until day 35, when it was removed. No chemical treatment against Varroa was applied in this group. For the implementation of the simplified trapping comb method, the queen was also caged on a single comb but remained there for 20 days. At that time, the comb, full of sealed brood, was removed, and the queen was transferred to a cage as used in the queen caging experiments in order to avoid oviposition in the brood nest. On day 25, after all

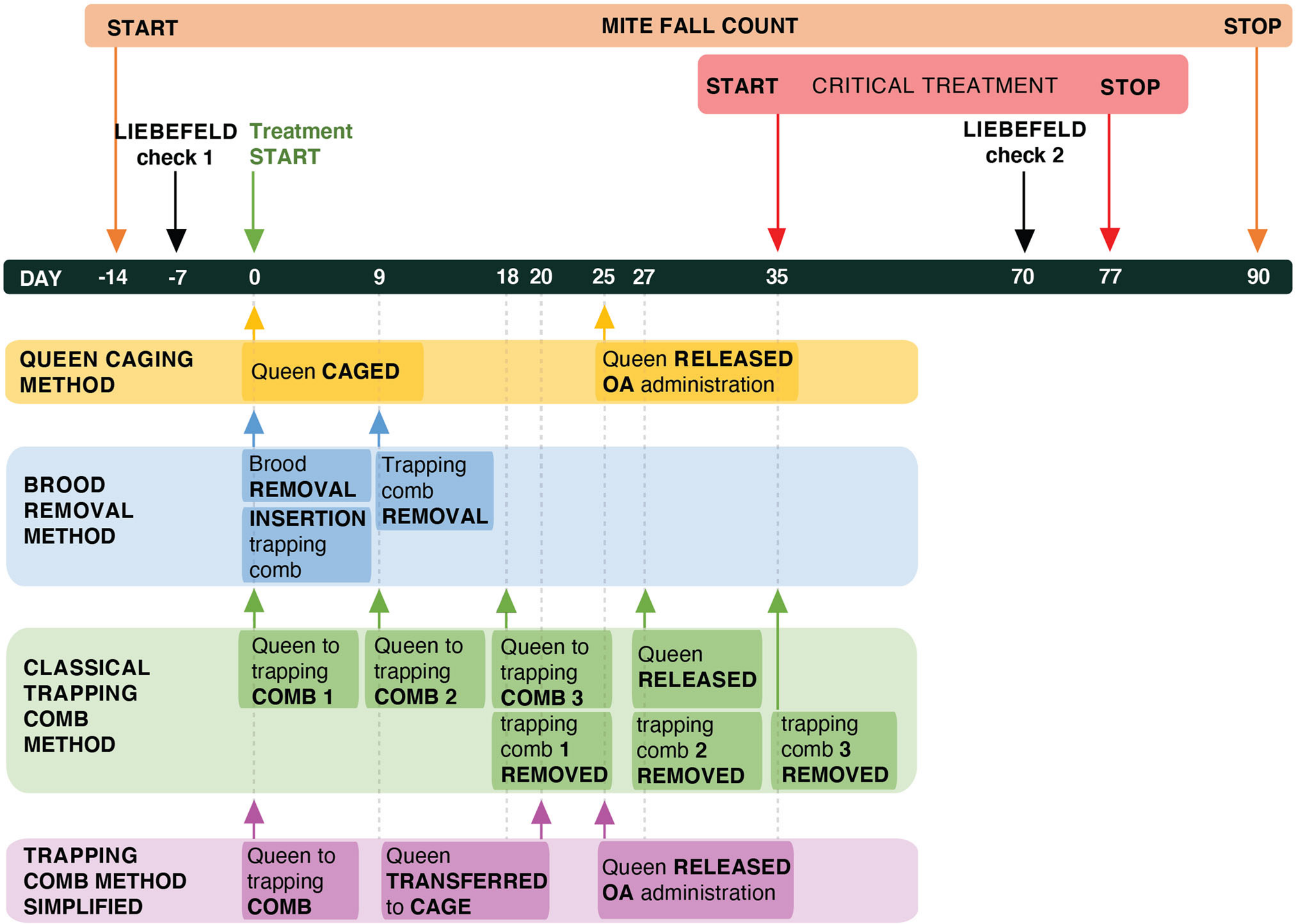

Figure 2. Timeline of the experiment. Four main methods are shown: queen caging (gold), brood removal (blue), classical trapping comb method (green) and simplified trapping comb (violet). The third Liebefeld check (not shown) was performed pre-winter but has no firm time point.

drone brood emerged, the queen was released, and 5 ml of 4.2% oxalic acid per occupied comb were applied like in the standard treatment group. For the Total brood removal method application, all brood combs were removed on day 0, with the exception of a single frame with open brood that remained as a trapping comb for Varroa. It was removed on day 9 when all brood was capped. Like in the trapping comb classic method, no chemical treatment against Varroa was applied in this group.

To investigate the number of surviving mites, all colonies received a treatment with either Apistan®, Apivar®, Apitraz®, Bayvarol®, or CheckMite® strips on day 35 after the start of treatment for a period of 42 days (critical treatment), according to label instructions. Mite fall on sticky bottom sheets, in order to avoid removal or dispersal of mites from other predators, was regularly counted from the start of treatment (day 0) until 14 days after the critical treatment (day 90) (Calderone, 1999). The data were used to calculate the efficacy of the queen caging protocols (cumulative mite fall before critical treatment against the total mite fall until 14 days after its end) and of the trapping comb treatments. Tested and critical treatments had to be synchronized between all groups in the same apiary. The starting date in each place was set up depending on the honey harvest that had to be finished before the treatment with oxalic acid on day 25.

The survival of queens and colonies was monitored until the following spring. To check for effects on colony development, the number of adult honey bees was estimated according to the Liebefeld method (Imdorf et al., 1987, 2019) before and 70 days post treatment. The ratio of the number of bees on day 70 to the number of bees before treatment was used to evaluate bee susceptibility and effects of the different treatment methods on colony development.

Finally, to compare the workload of the different methods, the working time needed per hive for performing all activities related to each treatment was measured.

Several workshops and field trainings were organized to standardize and harmonize the methodology among the research teams involved.

The data were analyzed by a SPSS-GLM ANOVA model, specifying the location (apiary), the season (year) and the treatment as fixed factors. Adjusted means were used to compare the efficacy of different

Table 2. GLM analysis for efficacy and relative colony strength 70 days after the treatment. Method, Location, and Year and interaction Method*Location were modeled as fixed factors.

	Efficacy of 4 queen caging methods			Relative colony strength 70 days after the treatment		
Source	df	Mean Square	F	df	Mean Square	F
Model	31	69475.666	392.420**	31	3.258	80.279 **
Method	3	3672.035	20.741**	6	0.078	1.923
Location	14	1047.450	5.916**	10	0.406	10.015 **
Year	1	22.456	0.127	1	0.388	9.553 **
Method * Location	12	602.155	3.401**	13	0.061	1.514
Error	268	177.044		279	0.041	
Total	299			310		
	$R^2 = 0.978$ (Adjusted $R^2 = 0.976$)			$R^2 = 0.899$ (Adjusted $R^2 = 0.888$)		

treatments by Bonferroni post-hoc test. In comparison of post-treatment infestation rates, mite fall data were log-transformed to reach normality and one-way Anova was used to determine differences between methods. Figures were designed in Python3 with Seaborn and Matplotlib packages, and the map was created using the Geopandas package.

Results

The efficacy of the oxalic acid treatment after queen caging was significantly affected ($p < 0.01$) by the method used, by the location and by the interaction of method x location, but not by the year (Table 2). The average adjusted mean efficacy of the standard 4.2%oxalic acid method was 89.57 ± 1.24% (mean ± SE), and ranged from 99.84 ± 4.41% to 70.94 ± 3.34%. In 10 out of 15 locations, an efficacy of higher than 90% was recorded (Figure 3). The efficacy of the Sublimation method was similar, with an adjusted mean value of 88.25 ± 1.79, and ranged from 98.78 ± 4.21% to 72.37 ± 3.58%. The adjusted mean efficacy of the 2.5% oxalic acid (8 ml) was 80.60 ± 2.16%, with the highest efficacy of 98.29 ± 4.41 and the lowest of 67.63 ± 4.88. The application of the 2.5%oxalic acid (5 ml) method showed the lowest efficacy (48.16 ± 3.25%) with a range from 39.74 ± 4.41 to 56.58 ± 4.41. The highest efficacy was achieved with the 4.2% (5 ml) and Sublimation methods, which were significantly more effective than the application of lower concentrations of oxalic acid (Figure 4).

The efficacy of TC Classic, TC Simplified, and Brood removal methods were compared to the standard 4.2% treatment by the post-treatment infestation levels. The highest mite fall after the critical treatment was observed with the TC Classic method and the lowest with the Brood removal method (Table 3), however, these differences were not significant (F (3, 221) $=$ 0.916, $p = 0.434$).

The adjusted mean of the relative colony strength 70 days post treatment was 0.544 ± 0.016, meaning that 54.4 ± 1.6% of bees were present in the colonies 70 days after the beginning of the experiment. The strength was significantly affected by location and year, but not by the different treatment methods (Table 2). In tendency, the lowest relative colony strength was recorded in 2.5% (5 ml) method and highest in TC Simplified and Sublimation(Table 4).

No colony losses due to treatment-related weakness or mite infestation were observed during the following winter period.

During our experiment, the highest workload was needed to perform the TC Classic and Brood removal methods, while the Queen caging with trickling and Sublimation were the least labor intensive ones (Table 5).

Discussion

Several biotechnical techniques have been developed to systematically interrupt the mites' reproductive cycle, or to remove a significant number of mites without swarming. The first of these methods to be published was the 'trapping comb' method (Maul et al., 1988), where the queen is sequentially caged on 3-4 combs for about 28 days, which are removed together with the invaded mites after the brood is sealed. To simplify the procedure, some beekeepers reduce the number of trapping combs, or even place the queen in small cages without any comb. To achieve a temporary brood interruption, these methods are meanwhile widely used by small and also by large-scale beekeepers in a few countries, like Italy (Allais et al., 2010; Pietropaoli et al., 2010) and Germany (Büchler & Uzunov, 2016). While the classical trapping comb technique can be applied to control Varroa mite infestation without the use of any drugs, the simplified technical measures are usually combined with a single oxalic acid (Nanetti et al., 2011) or thymol application (Giacomelli et al., 2016), which are known as a very effective treatments against Varroa mites in broodless colonies (Nanetti et al., 2003; 2011). Moreover, the application of varroacides with the temporary brood interruption technique allows avoiding the negative effects of the treatments on brood. Those techniques can thus help beekeepers to avoid the use of synthetic drugs, which is especially relevant for organic producers. In the EU, the use of organic acids is in general encouraged, as they do not leave any relevant residues to affect the quality of hive products, neither in a sensory nor in a quantitative way (Bogdanov et al.,

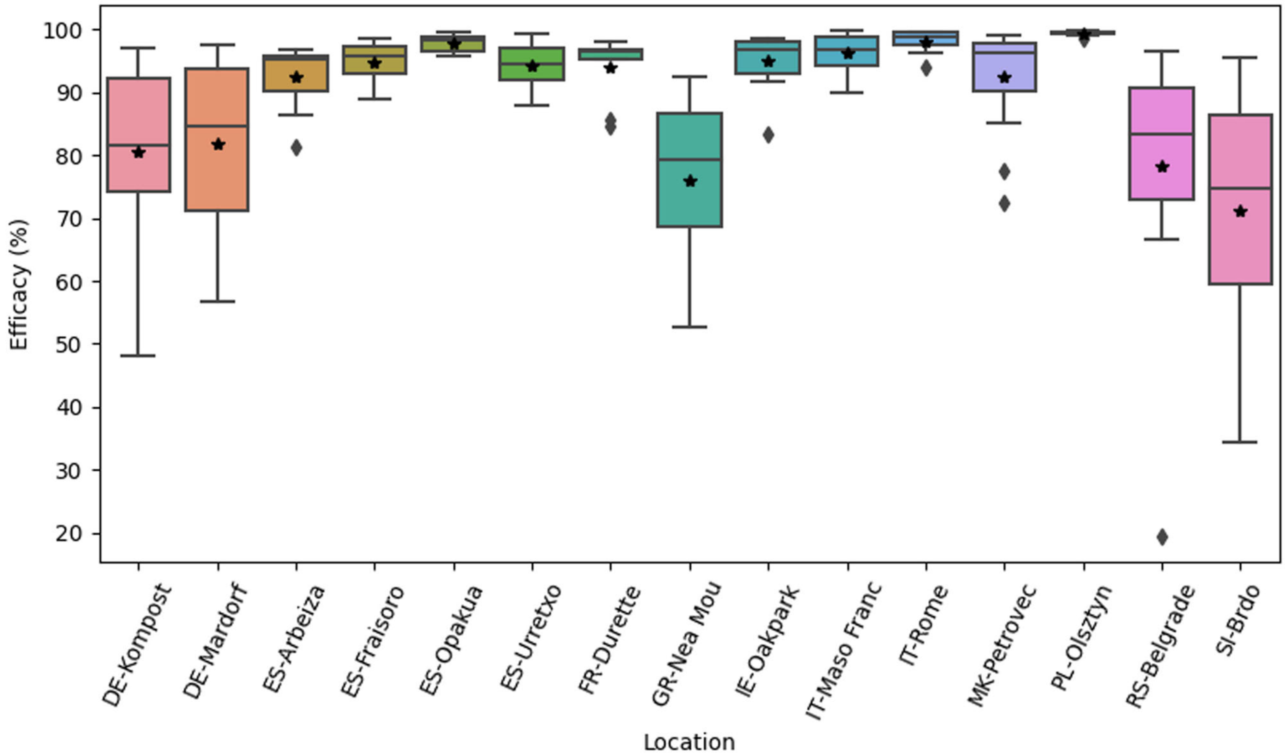

Figure 3. Efficacy of queen caging and administration of 4.2% oxalic acid by locations in years 2016 and 2017. Refer to Figure 1 for the apiary abbreviations. The horizontal line in box shows median. The box shows 1st and 3rd quartile. Whiskers extend to 1.5 x inter-quartile range (IQR). Diamonds mark the outliers, outside the 1.5x IQR. Additionally, the asterisks show mean values.

Table 3. Average mite fall per colony after critical treatment (mean values with 95% confidence interval).

Treatment	Mean	Lower CI-95%	Upper CI-95%
4.2%	181.10	126.98	235.22
TC Classic	248.21	97.72	398.69
TC Simplified	224.00	131.45	316.55
Brood removal	68.10	58.84	77.36

2002; European Parliament, 2018; Moosbeckhofer et al., 2003).

In our study, we included three known methods of brood interruption and tested them for the first time on a wide geographical range throughout Europe in order to compare them and to understand the best option to suggest to beekeepers. The results achieved in our study confirm their high potential for an efficient and timely mite infestation control. Trickling of 5 ml of a 4.2% oxalic acid solution (Apibioxal ®) per occupied comb, applied as a standard treatment in all research apiaries, was highly effective and reliable in most of the apiaries. The effect of lower concentrations of oxalic acid proved to be less efficient. This is in accordance with the findings of Gregorc et al. (2016) who achieved only 24% of mite mortality by trickling a 2,9% oxalic acid dehydrate solution on broodless colonies. No statistical difference in efficacy was found between trickling 5 ml per comb of a 4.2% oxalic acid solution (89.57 ± 1.24%) and sublimating 2 g of crystalline oxalic acid (88.25 ± 1.79%). Similar results were achieved in

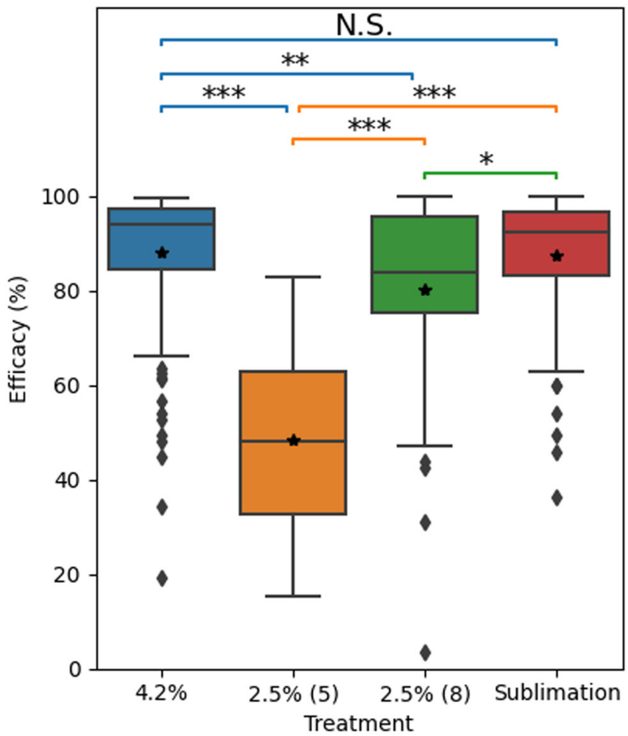

Figure 4. Efficacy of different queen caging methods (refer to Figure 1 for abbreviation). Bonferroni post-hoc significant differences between methods are marked. The horizontal line in box shows median. The box shows 1st and 3rd quartile. Whiskers extend to 1.5 x inter-quartile range (IQR). Diamonds mark the outliers, outside the 1.5x IQR. Additionally, the asterisks show mean.

Table 4. Relative colony strength 70-day post treatment as the percentage of bees that remained in colony (Adjusted mean values ± SE, 95% confidence intervals).

		95% Confidence Interval	
Method	LS mean ± SE	Lower Bound	Upper Bound
4.2%	0.529 ± 0.020	0.489	0.568
2.5% (5)	0.482 ± 0.051	0.382	0.581
2.5% (8)	0.508 ± 0.035	0.439	0.577
Sublimation	0.634 ± 0.036	0.564	0.704
TC Classic	0.522 ± 0.039	0.445	0.599
TC Simplified	0.652 ± 0.040	0.573	0.732
Brood removal	0.451 ± 0.068	0.318	0.584

Table 5. The average workload in minutes per colony for implementation of different methods.

Method	Mean	N	SD
4.2%	18.93	110	5.29
Sublimation	23.00	30	1.44
TC Classic	39.65	26	22.15
TC Simplified	14.11	18	1.02
Brood removal	37.00	10	0.00

other studies during winter (Coffey & Breen 2016). Finally, the efficacy of the trapping comb (TC Classic and TC Simplified) and the total brood removal (Brood removal) methods, measured by the mite fall after the critical treatment, were comparable to the standard 4.2% treatment. While the strong reduction of mite infestation in TC Classic and Brood removal groups was achieved without the use of any drugs, we speculate that a combination with oxalic acid might even improve their efficacy.

While beekeepers may worry about the negative effects of long-term caging on the queen, no increased mortality of queens due to the caging treatment was recognized during the two seasons of the experiment. However, even if such losses would happen, they could easily be remedied during spring and summer by introducing a new queen, while queen losses during late summer, fall or wintertime often result in the loss of the colony. Nearly all queens survived the caging period in good condition (data not shown) and resumed normal egg-laying soon after release, similarly as previously reported by Rivera-Gomis et al. (2017). The development of the experimental colonies usually met the normal local expectations, with some significant environmental effects by apiary location and season. Gregorc et al. (2017) did not find a significant increase of bee mortality after trickling 3.0 – 3.7% oxalic acid solutions compared to non-treated control bees under laboratory conditions. However, we noticed a slight tendency of a better bee tolerability of oxalic acid application by sublimation than by trickling (also reported by Al Toufailia et al., 2015), but in the end there were no significant differences between any of the treatments (see Table 4). Interestingly, even total brood removal did not result in a lasting weakening of the hives, which was well compensated, according to beekeepers' expectations, within about two months after treatment.

An optimization of the timing of the queen caging technique during the year is reported by Lodesani et al. (2019). Varroa management with the queen caging technique during the early season (late winter/early spring) was able to reduce the infestation rate without causing any negative repercussions on the honey harvest or seasonal colony development and performance. Moreover, queen caging can be used as a technique to control the colony development during the year, for different reasons, such as to regulate the development of the colony according to nectar flow or to control swarming (Forster, 1969; Simpson, 1958). Even if such practice appears counter-intuitive at first sight, it was shown that simple brood interruption practices, such as colony splitting can decrease winter losses in the US (Haber et al., 2019). Brood management (removal/redistribution) is therefore a crucial aspect of proper colony management, as it is related to Varroa population dynamics. The timely caging of the queen, considering the bloom of the main summer flow, could free part of the bees' work force from taking care of the brood to engage in nectar collection instead. Unfortunately, the effects of the tested techniques on honey productivity could not be measured in our study. A positive effect of brood removal during the main flow on the honey harvest within the following 14 days was reported from Germany (Büchler & Uzunov, 2016), but regional and seasonal differences may have to be regarded. Thus, additional research is needed to better understand the effects of queen caging and the presence of brood pheromones on honey production and to optimize the timing of brood interruption under local conditions.

One major factor limiting the widespread adoption of these methods might be the time needed to locate the queen and the individual level of experience required to handle the queen. In our study, with a heterogeneous group of researchers with different levels of experience in beekeeping and under variable beekeeping conditions, a realistic average estimation of less than 20 minutes is given for the working time needed to apply the 4.2% method. Still, beekeepers, who want to avoid any search for the queen, may decide to use the Total brood removal technique although it is more labor intensive.

There are some technical challenges linked to the brood interruption methods. One aspect is the risk of robbery, mainly during nectar dearth. Especially the total brood removal, requiring the transfer of many combs between boxes, bears the risk of attracting foragers from neighboring colonies. As suitable precaution measures, the number of colonies per apiary should be kept as low as possible, and hive manipulations should preferably happen during periods without intensive flight of bees (f.e. in the early morning, at the end of the day, or during rainy periods). Another aspect is the further use of the brood combs removed during Brood removal and Trapping comb methods. They can either be stored in brood collecting colonies or, as a less time-consuming option, could simply be melted to harvest the wax. To avoid robbery, the brood collectors should be placed in a separate apiary outside the flight range of the donor colonies. As soon as all bees emerge, the brood collecting colonies can be treated with drugs in the usual way. In general, one colony out of two to three donor colonies can readily be built up this way.

The selection of the most suitable method depends on the individual skills, the apiary size, the available working time per hive, and any specific production goals of the beekeeper. Total brood removal is probably the method of choice for beekeepers who want to avoid any search for the queen or want to combine the treatment with a buildup of additional colonies. Total brood removal combined with the use of a trapping comb and also the Classical trapping comb technique is of special interest to organic producers, who are aiming at minimizing the use of drugs. On the other side, large-scale operations may prefer the simplified trapping comb or the caging technique in combination with an application of oxalic acid, as those methods are less time consuming. Given the similar efficacy of trickling and evaporation, the choice of the oxalic acid administration method mainly depends on the national legal registration status, the experience of the beekeeper, and the cost of the treatment per hive.

Beyond a short-term potential of the tested seasonal brood interruption methods to reduce colony winter losses, they may additionally contribute to a long-term solution of the Varroa challenge. A consequent application of seasonal brood interruption and mite control bears the high potential to dispense with the need for winter treatments. This is supported by long-term experience with the management of drone colonies without winter treatment in several mating stations of the German breeder association "Arbeitsgemeinschaft Toleranzzucht" (Büchler et al., 2010) as well as by the results of Seeley and Smith (2015), who identified low drifting (DeGrandi-Hoffman et al., 2016; Nolan & Delaplane, 2017) and a reduction of mite infestation during the process of natural swarming as prerequisites for the successful wintering of colonies without treatment.

Varroa infestation during the pupal stage is known to reduce the development of spermatozoa, the flight ability, and the life expectancy of drones (Bubalo et al., 2005; Duay et al., 2003). Differences in mite infestation do therefore affect the fitness of drones and result in a higher reproduction rate of less susceptible colonies (Büchler et al., 2006). We suppose that this is a major reason why natural selection in non-treated populations favors mite-resistant colonies, as seen by the fast development of mite resistance in several untreated, nature-like honey bee populations around the globe (Locke, 2016). Opposite to this, regular winter treatments may overrule this natural selection effect and instead support the reproduction of the most intensively treated colonies. A general switch from therapeutic treatments during winter to mite control based on brood interruption during summer may thus contribute to a wider establishment of locally adapted, resistant stock and sustainable Varroa control.

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No potential conflict of interest was reported by the author(s).

Supplementary material

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