# The efficiency of Drone Brood Removal (DBR) + Lactic Acid Treatment (LAT) to control the Varroa destructor infestation.

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## **Abstract**

Varroa Destructor is one of the greatest threats to the beekeeping industry based on Apis *mellifera* and without treatment colonies will most likely die within 2 years. Several studies have shown that Drone Brood Removal (DBR) is efficient in reducing the mite population although additional treatments are usually needed. In this study we demonstrate that with an improved drone comb, I.E a three-way comb where fresh drone cells are offered weekly, there is a high efficiency in the mite removal and with additional Lactic Acid Treatment (LAT) the mite population can be supressed to a level that allows for a healthy colony



Three-way drone comb where one division is ready for removal.

during the whole season. DBR+LAT treated colonies were compared with control colonies treated with "standard" Varroa management methods based on organic acids (Formic Acid and Oxalic Acid). The mite drop in the Control group increased 25-fold from May to end of August (prior to the Formic acid application) while that in the Test group only increased by a factor of 1,3. Since natural mite drop is a rough indicator of the total mite population it indicates that more than 90% of the expected mite population development was avoided due to the DBR+ LAT treatment. After the Control group received the Formic Acid (FA) treatment the daily average mite drop showed no significant difference

## Abbreviations used:

FA= Formic Acid

OA=Oxalic Acid

LA=Lactic Acid

LAT=Lactic Acid Treatment

DBR=Drone Brood Removal

between the groups although a greater variance in the control group could be noticed indicating that some control colonies retained very high mite populations.

In the last week of November, when the hives were brood less, all hives received an Oxalic Acid (OA) treatment to check the final mite population which showed no significant differences between the groups. The data support that DBR + LAT treatment is a viable method for varroa management, comparable to the "standard" treatments with formic and oxalic acids. In addition, we found a positive linear correlation between daily mite drops and the total mite population in November and hence the average daily mite drops can be used to decide, on a colony-to-colony basis, whether any autumn treatment is needed or not.

# 1. Introduction

The parasitic mite Varroa destructor is considered by many researchers and beekeepers to be a major threat to honeybee colonies and consequently a threat to the pollination business wherever beekeeping is based on Apis *mellifera*. When the mite appeared in Europe the first strategy included Xeno chemical treatments such as Apistan (fluvalinate) and CheckMite+ (Coumaphos), which showed good efficiency. However, miticide resistance is now a widespread, and increasing, problem (Baxter et al. 1998; Elzen et al. 1998; Elzen and Westervelt 2002). In addition, miticide residues can be found in wax, and honey, many years after the chemical has been used (Karazafiris et al 2016). Organic acids, such as FA and OA, are recent products used to control the mite infestation. Since they are water soluble, they don't tend to be absorbed by the wax, but they can contaminate the honey and thus they should be used outside the nectar flow period. The consequence is that since the treatment must be

delayed until the flow is ended it may allow the mites to reach levels that results in serious damaged colonies (DeJong 1990, Amdam et al. 2004).

Another issue with FA is that the efficiency varies with ambient temperature and can be anything between 51-95% (Steube et al, 2021). OA, on the other hand, is only efficient on brood less colonies (Gregorc & Planinc, 2012) and thus a combination of treatments (FA+OA) or multiple treatments may be needed. Furthermore, FA and OA can have negative impacts on the colonies, such as increased brood and adult bee mortality and can, in some cases, even cause queen mortality (Rosenkranz et al 2009, Thielka 2017). Gunes et al, 2017 showed that FA and OA treatments caused an increase in the level of stress protein, HSP 70.

LA appears to be less harmful to the bees but still have high efficiency - over 90% on brood less colonies (Kraus et al. 1994; Domatskaya, T.F, Domatsky, A.N. 2020).

The sustainable long-term solution is mite resistant stocks, which are already available (Harbo and Harris 1999, 2001, 2003; M Oddie et al 2021), but their performance has been non-consistent, and it may require many years to incorporate them into the global honeybee population. Therefore, colonies will require supplemental control measures for many years to come to keep the yearly colony losses at a reasonable level.

Consequently, a method that maintains low mite levels during the summer and early fall, to protect the colonies until the end of the flow season, is needed. The method should preferably maintain the low mite level until brood rearing ceases and the mites no longer are able to reproduce. The reproductive behaviour of V. destructor suggests a non-chemical method for suppressing mite populations. Mites reproduce on their host's immature stage and those that reproduce on drone brood average 2.2-2.6 female offspring per host, while those reproducing on worker brood average 1.3-1.4 female offspring per host (Martin 1994, Martin 1995, Fuchs 1992). Differences in fecundity are correlated with the duration of the capped stage of each host type, which is greatest in drones, intermediate in workers, and shortest in queens (Jay 63). Female mites mirror the reproductive opportunities given by the different host types and thus drone brood is often infested at a higher rate than worker brood, with average differences between 5 and 10 -fold (Fuchs 1989 and 1992; Boot et al 1995; Schulz 1984). The prevalence, however, is dependent on the mite population and available drone cells (Fuchs 1992. Consequently, if capped drone broods are removed from an infested colony, many mites may be removed without negative impact on the size of the worker population, and mites with the greatest fecundity are removed.

Several authors have conducted DBR studies showing high efficiency of the treatment (Calderone. N. W, 2005; Charriere, 2003; Odemer et al. 2022). Although the efficiency showed to be high complimentary autumn treatments were still needed although Calderone find that some colonies, on a colony-by-colony basis didn't need any autumn treatments. In these studies, one drone comb per hive was used and the whole drone comb was cutout monthly (Calderone and Odemer) or irregularly (Charriere). Since the window of opportunity of the drone brood is limited the efficiency of the method could be improved if mature cells would be offered weekly. Therefore, the goal of this study is to determine if natural mite drop and final mite population in colonies treated with DBR (using a three-way drone comb where one portion is cutout every week) in May-June plus LA sprays in July-October matches to that of colonies receiving the "standard" treatments of FA in August and OA in December. Secondly, we want to show that with this method it is possible to keep daily mite drop on a low level over the season which would indicate that also the total mite population is kept on a level that is below the detrimental threshold for the full colony.

#### 2. Material and Methods

## 2.1 Experimental field sites and colonies

27 Experimental colonies of locally adapted Apis Mellifera were kept in 7 apiaries in Kungsbacka and Alingsås on the west coast of Sweden. The bees were kept in double brood chambers with 10 standard LN frames (370x220 mm) in each chamber. The natural mite drop was measured in April 2023 and any hive with an average daily mite drop greater than 2 mites per day were treated with 5-8 ml of 15% lactic acid applied by spraying directly on to the bees. After one week the daily average mite drop was measured and the lactic acid treatment was repeated if needed until all hives had a daily average natural mite drop below 2 mites per day. 3 out of 11 test colonies needed LAT due to high starting value of the Mite drop

Standard management practices were then used for the reminder of the season, including the addition of honey supers above a queen excluder, and removing full combs of honey.

The colonies were organized in matched pairs randomly divided into positive **Control colonies** receiving formic acid in August (40 ml of 60% formic acid on Wettex cloth at the top of the frames applied for one week) and oxalic acid once the colonies were brood less at the end of November (applied by trickle method with 3,2% oxalic acid dissolved in 50% sugar syrup. **Test colonies** receiving multiple drone cut-outs in May-June plus lactic acid sprays when a colony's average daily mite drop exceeded 2 mites during July through October.

In total, 11 matched pairs and an additional 5 test hives were used. All hives received one three-way drone comb, (fig. 1), kept in the upper brood chamber placed next to the last worker brood comb. In the control group, drone combs were left in place throughout the season, but lifted and returned the same way as the test colonies to simulate the manipulation of the test colonies. The bees were left to use the drone combs in the test colonies as they pleased (for brood rearing or to store honey).



Fig 1. Three-way drone comb where the middle division is capped and ready to be removed, the right division is partly capped and will be ready the coming week and the division to the left is filled with newly laid eggs.

**Mite drops count:** The mite drop of all colonies was checked weekly and the count was divided by the numbers of days, I.E 7, to calculate the average daily drop.

# 2.2 Application of treatments

## 2.2.1 Drone brood period May-June

On day 0, a three-way drone brood comb without foundation was placed into the upper brood box of every hive adjacent to the worker brood. On day 7, division 2 and 3 of the drone comb with or without eggs and larvae, were cut out. On Day 14, division 3 was cut out, so at the end of week 3 there were three different age cohorts of drone broods in each of the three sections. From day 21, and then every 7<sup>th</sup> day, the section with closed brood cells was cut out and the cut-out comb pieces were placed in the freezer, labelled by date and hive number, for later dissection.

## 2.2.2 After drone brood period July-mid October

**Test colonies:** If the daily average mite drops of a Test colony exceeded a threshold level of 2 mites per day a LA treatment of the brood chambers was applied according to the manufacturer's instruction – 5 ml of 15% LA dissolved in water on each comb applied by spraying directly on the bees. After one week the mite level was checked and the LA treatment repeated if needed.

**Control Hives:** If the daily average mite drops of a Control colony exceeded the upper threshold level of 7 mites per day, it was counted as a "fail," and dealt with accordingly.

To all Control hives August treatments of formic acid treatment were applied (40 ml of 60% formic acid on Wettex cloth at the top of the frames).

# 2.2.3 Final mite population determination

Once the colonies were brood less, an oxalic acid trickle of 5 ml of 3,2 % oxalic acid dissolved in 50 % sugar syrup per frame interspace containing bees, was applied to all Test hives and Control hives. The total number of mites that dropped onto the sticky board over the next seven days was counted.

#### 2.3 Data collection

Cutout of drone comb divisions started in early May and continued to the end of June and the cutout divisions were marked and frozen for later examination. From the frozen drone cakes, 100 closed drone cells were opened from each division (determined by counting) and the number of cells that were infested by a foundress mite was recorded, see fig 2, which gave the infestation level of the drone comb. In our view it is better to count foundation mites, I.E infested cells, rather than total mites in the drone cells since it is difficult to determine if the counted mite is in fact an invading foundation mite or an offspring. In addition, it is difficult to know if there were 1, 2, 3, 4 or 5 mites per drone cell and hence we do not know what we measured if the total number of mites is counted. When foundation mites are counted, we know how many invading mites have been trapped, we can calculate the infestation rate in the drone comb, and we can also estimate the number of mites that we have stopped from re-entering the colony.

The total number of drone cells in the cutout division was estimated by measuring the area of closed drone cells and divided with the measured area of 100 cells (in our case 68x32 mm) which allowed us to approximate the infestation rate of the drone brood. The total number of trapped foundation mites were then estimated by multiplication of the infestation level with the total number of closed drone cells. The area of the capped drones in each division was measured and divided with the area of 100 cells to calculate the number of cells in each division. The number of capped cells (Cc) is calculated with the following formulae:  $Cc=A_d/A_{100}x100$  where  $A_d=$ area of the cutout division,  $A_{100}$  is the area of 100 cells (68x32mm).



Fig 2. Opening of drone cells and counting invaded cells, I.E. foundress mites.

#### 2.4 Statistical analysis

Microsoft Excel data pack were used for the statistical analysis and the p-values between test group and control group were calculated by Kruskal-Wallis test due to uneven group sizes and variances being different between the groups. Students t-test was used to calculate the p-value for results within the same test group.

## 3. Results

Of the initial 16 **Test colonies** 6 were excluded due to swarming or split of the hives to prevent swarming. Of the 11 **Control colonies** 1 was excluded due to an extreme increase in the mite drop (>>7/day) and had to be treated with drone brood removal to decrease the level, two more hives were excluded due to swarming. In Fig. 3 the timeline of treatments is shown for some Test colonies.

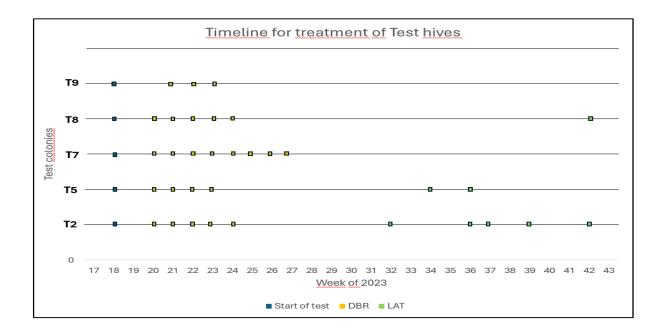


Fig 3. Timeline of treatments for some of the Test colonies (one from each apiary). W 18 is the start of the test (1st week of May). DBR takes approximate 1 m to apply, and LAT is a 3-4 m operation. The respective Testy hive numbers are found in fig. 9.

#### 3.1 Average daily mite drop

As shown in the graph below, Fig 4, the daily average mite drops were lower in all test colonies with little variance at the end of the Drone cut out. The difference between Test hives and Control hives was substantial but not significant (p>0,1) with  $0.2 \pm 0.1$  and  $2.4 \pm 1.4$  daily average mite drops respectively. At the end of August (prior formic acid treatment) the difference was significant (p<0,05) with  $1.2 \pm 0.4$  and  $7.6 \pm 3.1$  daily average mite drop respectively. The change in average mite drop for the Test group before and after DBR was significant (P=0,011) with starting value of  $0.9 \pm 0.2$  and  $0.2 \pm 0.1$  after the DBR was finished.

From May to end of August the Test group only increased the average daily mite drop by a factor of 1,3 (from 0,9=>1,2 mites per day), still below the threshold value for healthy colonies, while there were a 25-fold increase in the Control group (from average 0,3=>7,6 mites per day). Based on the 25-fold increase for the Control group the results for the Test group indicate that 90-95% of the expected mite population increase, from May to end of August, had been avoided. Once the FA treatment was applied the daily mite drop decreased for the Control hives but with greater variance than Test hives which continued during the autumn. The final Oxalic treatment results, see Fig 6, showed no significant differences between the final mite populations of the two groups ( $228 \pm 80$  and  $229 \pm 100$  mite drops for Test and Control respectively). It should be noted that two control hives did not receive any FA treatment, judgment call, and hence they are shown separately in the graph in Fig. 4 (light blue bars).

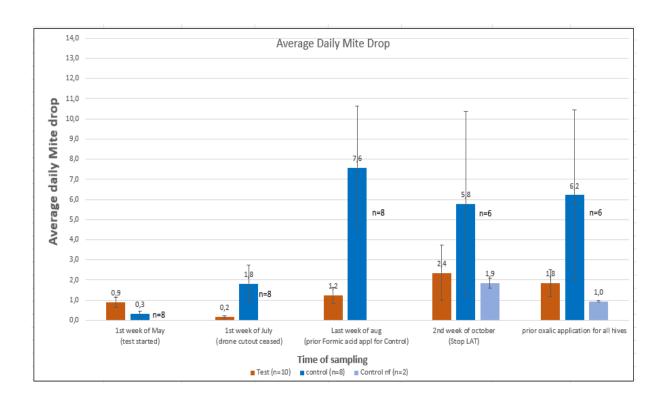


Fig 4. The graph shows the progression of mite drop counts; the start of the test with all colonies below 2 mite drop/day. The Control group showed big variances of the mite drop where especially two hives maintained high mite drop indicating that the FA was not very effective on these two hives. Two of the Control hives received no FA in August, a judgment call, due to low mite drop. They are plotted separately in light blue colour in October and November bars.

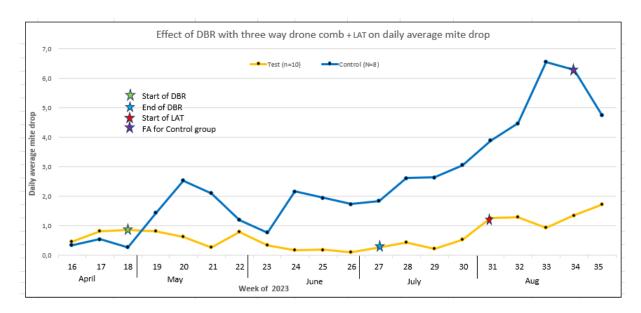


Fig 5. The graph shows the progression of mite drop counts for both Test and Control. The DBR supress the mite drop and with occasional LA applications the mite drop continued to be low during the whole season.

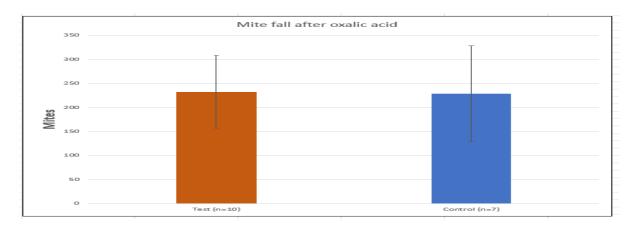


Fig 6. Estimated final mite populations per hive, based upon mite drop counts after oxalic treatment in all colonies. The difference is not significant, but the test colonies showed a lower variance than the control colonies where only 1 out of 10 test colonies had more than 400 mite drops whereas 2 out of 7 control hives had more than 400 mite drops after the oxalic acid treatment although it should most likely have been 3 out of 8. The Control hive with highest mite drop prior Oxalic acid (27,8 mites/ day) ended up in a storm that blow away all the mites after OA application and hence removed in this graph (n=7 for control).

## 3.2 Weight gain (honey yield)

Honey yield was measured by weighing removed honeycombs where the weight of the comb with foundation was deducted.

The weight of the Honey extracted was on average  $26.2 \pm 6.2$  and  $27.6 \pm 4.9$  for Test hives and Control hives respectively with no significant difference. The result varied between 0 and 52,7 kg for the test hives and between 5 kg and 56 kg for the control group, see Fig 7. The weather during the year started with sunny weather in May with good Rape seed nectar flow and continued with a draft through June. In July and August, the rain poured down and thus there were no nectar flow and hence only the hives that was strong early in the season managed to yield any mentionable amount of honey.

The result was thus mainly decided by the strength of the hives in early May since extraordinarily small amount of honey was yielded in June and onward.

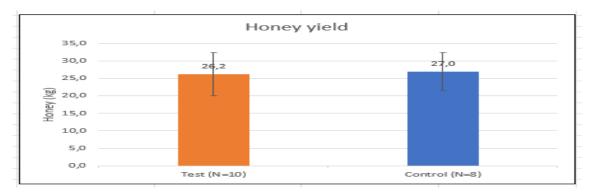


Fig 7. The graph shows the average honey yield for both groups with no significant difference supporting that DBR did not negatively affect the honey yield.

## 3.3 Drone cells prevalence

The mites have a high preference to enter a drone cell (Fuchs 1992) but if there are more mites than available drone cells then the mites enter a worker cell rather than share the drone cell with another mite. More than one mite per drone cell will lower the fecundity and therefore it's better optimization for the mite to infest a worker cell. From the result we see that we have a max average infestation level of 15 % (the highest value was 40%) so the available drone cells appear to be enough to offer each ready mite a fresh drone cell. The trapped number of mites was stable the three first cutouts (average 40-60 mites/ cutout) and then it dropped drastically from 4<sup>th</sup> cut and onwards, see Fig. 8. The daily Average mite drop decreased to close to zero which confirmed that the mite population had become very low.

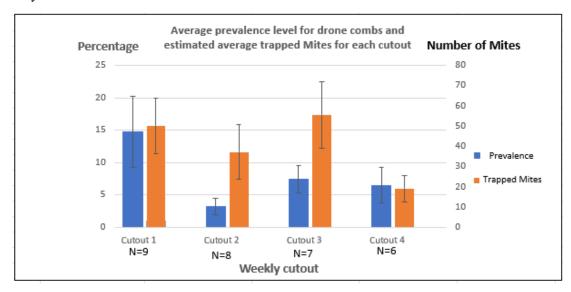


Fig 8. The graph shows the average prevalence level and the estimated average number of trapped mother mites in the respective cutouts.

3.4 Correlation between average mite drop (prior OA treatment) and estimated mite population in November.

All hives received OA treatment at the end of November (when colonies were brood less) to determine mite population after the treatments. The result was plotted against average daily mite drop (prior the treatment) and showed a strong correlation (y=123x with R<sup>2</sup>=0,9546, where y = total population of mites and x is daily mite drop), se Fig 9. The result supports that in November the mite drop can be used to judge the total population and thus judge if an additional treatment is needed. The result from this study confirms other researchers' results that a linear correlation exists (Brodsgaard, C.J, Brodsgaard, H.F., 1996). (Genersch et al, 2010) found that the threshold infestation level was 6 % to keep the winter losses below 10%, which gives 600 mites as max allowed winter population, if only strong colonies with minimum 10000 bees are considered to winter in. Smaller colonies than 10000 bees increase the risk of winter losses (Jeffree, E, 1956, Genersch et al, 2010). The results support that mite drops of < 3 mites per day (correlating to about 400 mites) can be tolerated in brood less colonies without any further autumn treatment, marked in the graph with green lines.

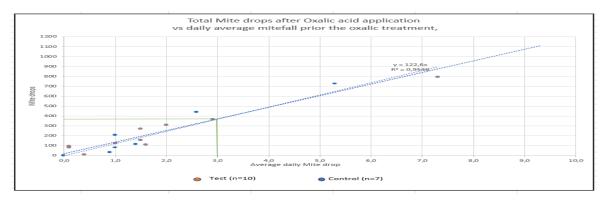


Fig 9. Correlation between total mite drop after OA treatment and daily average mite drop prior the treatment.

#### 3.5 Varroa mite count

In the graph, Fig 10, some data from the study is shown; In column 7 the trapped mites are given, and it varies from 0 to 379 mites showing that mite population varies considerably in the different colonies, indicating that some sort of resistance is present in some colonies. This is even more clear in column 8 where the total number of mites collected through the season for each colony including daily mite drop (with mite drop after Formic acid for Control group), mite drops after oxalic acid for all groups and trapped founding mites via DBR. In present study we counted infested cells (I.E founding mites) instead of the number of mites in the drone brood. The reason, as mentioned earlier, is that it is difficult to know how many of the counted mites are foundress mites and how many mites are mature offsprings and hence it is unclear what is measured. Consequently, it is difficult to compare the result from this study with other researchers result where for example (Charrier 2003) reported average of 788 mites/hive and (Odemer 2022) used median value and reported 271 mites per hive. Our result shows an average value of 136 foundress mites per hive (with high variance between the number of cutouts) and to get the real number of mites in the drones we have cut out we need to also add the offsprings. According to (Fuchs 1992) the Foundress mite will have 2,2-2,6 offsprings in a drone cell so we used 3,5\*the infested cells (2,5 offsprings+ the foundress mite) to estimate the equivalent number of mites that we have stopped from re-entering the colony, which gives an average of 476 mites per colony. All values for each hive are shown in column 14 and the results varies between 0 and 1327.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number	Hive	Group T (test) or C (control)	Mite drops/day (prior Formic treatm for control) last week of Aug	Nuber of lactic treatments	Total daily mitedrop (Until oxalic acid)	Total Mite drops Post oxalic acid,	Total DBR trapped Foundation Mites	total removed mites in hive over the year	Number of infested cells	Number of opened cells	Number of cutouts	Estimated Total DBR trapped Foundation Mites	Number of cutout drone cells	Estimated mites in the drone cutout (trapped x3,5)
T1	EM-T1	T	2,1	7	303	309	0	612	0	0	0	0	0	0
T2	EM-T2	T	2,0	4	416	368	138	922	58	500	5	138	1311	483
T3	EM-T3	T	0,7	0	114	110	100	324	33	100	1	100	305	350
T4	EM-T4	T	1,4	11	1000	795	379	2174	73	400	4	379	2582	1327
T5	Malin-T2	T	4,0	2	469	156	280	905	33	400	4	280	3393	980
T6	KJ4-T2	T	0,7	0	87	125	197	409	40	500	5	197	2742	690
T7	KJ5-T3	T	0,0	0	24	13	60	97	9	800	8	60	5724	210
T8	Åsa-T1	T	0,0	0	270	186	172	628	56	500	5	172	2724	602
T9	Mariedal T	T	0,3	0	316	84	38	438	4	300	3	38	2850	133
T10	Saxebäcken T	T	0,0	0	222	96	0	318	0	200	2	0	1900	0
C1	EM-C1	С	3,6	N/A	319	727	0	1046	NA	NA	NA	0	NA	NA
C2	EM-C2	С	4,0	N/A	241	438	NA	679	NA	NA	NA	NA	NA	NA
C3	Malin-C1	С	0,0	N/A	11	1	NA	12	NA	NA	NA	NA	NA	NA
C4	Malin-C2	С	4,3	N/A	591	206	NA	797	NA	NA	NA	NA	NA	NA
C5	KJ1-C1	С	21,4	N/A	1273	storm	NA	1273	NA	NA	NA	NA	NA	NA
C6	KJ3-C2	С	21,4	N/A	3106	113	NA	3219	NA	NA	NA	NA	NA	NA
C7	Mariedal C	С	4,3	N/A	287	84	NA	371	NA	NA	NA	NA	NA	NA
C8	Saxebäcken C	С	1,9	N/A	142	34	NA	176	NA	NA	NA	NA	NA	NA

Fig 10. The table show, for information, a summary of all mites that have been removed from each colony. Colony KJ1-C1 didn't report any result after OA treatment since a storm blow away the mites after the treatment. The colour in column 1 shows the apiary.

#### 4. Discussion

In this study we show that if a three-way drone comb is used the mite population is rapidly decreased and, with occasionally LAT treatment, it is possible to maintain the low mite population through the season. Of the test hives (n=10) none had a mite drop in august that require any FA treatment and only one had a mite drop in November that would require an OA treatment.

There seem to be a better long-term result in this study compared to earlier studies (Charriere, 2003; Calderone. N. W. 2005; Odemer et al. 2022) and we hypothesize that it is due to consistently present mature drone cells for 4-8 consecutive weeks and, by cutting the divisions weekly, it is avoided that any mites are mistakenly released by a too late cutout. In most studies the DBR is performed using one full frame which is cutout one time per month or every third week. The mites are only interested to enter a cell for 2-3 days prior the capping and if the comb is replaced monthly then there will be two-three weeks where no mites are trapped. During the times it takes to replace the comb and new cells have matured the mites will breed in other cells, enable the mite population to recover between each cutout. With the method presented in this study the mite trapping is conducted consistently up to 6 weeks in a row which will ensure that a maximum number of mites are removed, without a chance for the mite population to be rebuild between cutouts. Consequently, the mite population is supressed at the end of the drone season giving the mites a brief time span to grow, and in combination with the occasional LAT treatments the mite population can be maintained below the detrimental level through the season. Additional experiments/data is needed to determine if the result can be repeated also in other areas where the drone period is different – in our area (South Sweden) most of the drones are produced during 6-8 weeks from early May to end of June providing an ample opportunity to trap maximum mites during the period when they else would multiply.

The DRB treatment did not appear to have any negative effect on either colony development or the honey yield since no significant differences were found. Another crucial point is that not all drone cells were removed since scattered drone cells were found along the edges of most worker combs. We estimate that about half the drone brood was removed.

The final mite count after the OA treatment, carried out in late November, showed a strong correlation between average mite drop and total Mite population (y=123x, where y = total population and x is daily mite drop with  $R^2=0.9588$ ) which indicate that it is possible to use the mite drop in November to judge whether any late autumn treatment is needed or not.

Genersch et al, 2010 found that the threshold infestation level is 6 % to keep winter losses below 10% Based on their findings, and considering strong winter colonies only (> 10000 bees), it allows 600 mites as max allowed winter population, supporting the findings from this study that it can be recommended

that autumn treatment is not needed if the daily average mite drop in November is below 3 mites/day correlating to a total population of about 400 mites.

#### Cons and Pros with DBR+LAT treatment

The result of the study shows that DRB and LAT strategy is an efficient way to manage the mite population and keep it below the detrimental level for a healthy colony. It also offers a tool to determine if and when stronger chemicals are needed, and thus avoid the stress of unneeded treatments. Furthermore, the risk of a too late treatment, since FA and OA can only be used after the nectar flow is over) resulting in an extensive portion of damaged winter bees, is avoided.

It's also a very simple method that requires no special equipment nor any advanced technique making it comfortable for the new beginners to implement. Furthermore, it reduces the handling of dangerous chemicals since LA is a relatively harmless acid, especially since 15% solution is used. LA is used in the food industry for fermentation (Barbosa ET AL, 2017), in the beer industry and used as chemical peeling and hence considered safe to use. The downside of the method is that the DBR must be done weekly to be effective, and the mite drop must be checked regularly all season in order to detect any sudden increase. In the region where this study was carried out (south west of Sweden) the swarming season and drone brood season coincide and hence, provided that weekly swarm control is carried out, the added time, for DBR, will be the time it takes to cut out one section of the drone comb, which is a matter of a minute or two. The check of mite drop takes very little time if the mite fall is low (actual counting is only needed if close to, or above, the threshold value) it is a matter of pull out the board and carry out an assessment but must be done regularly (weekly or at least biweekly). The application of LAT can appear to be time consuming since the recommendation is to pull out each comb and spray them individually and this process takes about 10 minutes. However, we have developed a faster way to apply the spraying where we part two adjacent combs and spray each side with the same amount of solution (in our case it is 3 sprays per side to get the 5-8 ml/ side, see Fig 11). With this application method the time needed is 3-4 m per hive and application.

As described the method require regular monitoring and extra work in the cases where LAT is needed but due to the simplicity of the method, where no special tools or advanced technique are needed, it should fit most hobby and part time beekeepers (<50 hives).

When the colonies were brood less, in late November/early December we found a positive straight-line correlation where the average daily mite drop x 124 would estimate the total population. The straight-line correlation confirms the result of other studies (Liebig,1983; Brodsgaard, C.J, Brodsgaard, H.F., 1996).



Fig 11. LAT where the combs are mowed sideways, instead of removing and spraying each comb individually, and the solution is sprayed between the combs. We have not noticed any difference in the efficiency, so the two methods appear to be equal.

# Time needed to carry out the treatments:

DBR can be included in the weekly check of the hives (swarm control, removal of honey, adding super etc). The extra time needed to lift the comb, brush of the bees and cutout the part is approximate 2 m/cutout (4-6 times per hive, carried out weekly).

The LA application takes 3-4 m per application if the method, as described in Fig 11, is used. 2-4 LA applications per colony can be considered as normal although it varies.

## **Conflicts of interest:**

There are no conflicts of interest to be declared.

# **Acknowledgements:**

We would like to thank the team that meticulously carried out the time-consuming data collection and by that hard work enabled this study to be carried out.

Åsa Henry, Karin Gransvik Nilsson, Karl Johan Holmstrand, Staffan Görander, Jerker Andersson, Malin Sundelin

A special thanks to Randy Oliver, who functioned as mentor and technical advisor.

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