

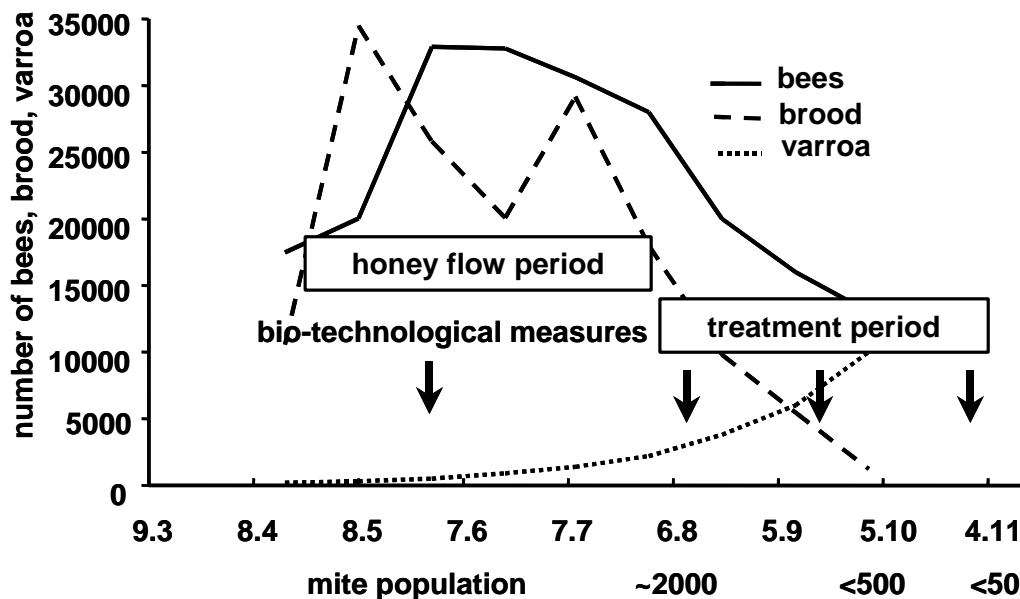
# Alternative strategy in central Europe for the control of *Varroa destructor* in honey bee colonies

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The bee parasite *Varroa destructor* has developed resistance against traditional varroacides in many regions of Europe<sup>46</sup>. Of course, there are substitutes, such as organic acids and components of essential oils. However, these are effective only, if applied in the frame of a control strategy. The aim of this article is to provide more background information on the different steps of this alternative treatment strategy, developed over the past 15 years.

## WHAT FACTORS HAVE TO BE CONSIDERED FOR THE ESTABLISHMENT OF A CONTROL STRATEGY?

The climate conditions, the periods of honey flow, the way of beekeeping and the development of the *Varroa* population determine the treatment strategy with the aim to keep the population of *Varroa destructor* below the damage threshold in honey bee colonies. Thus, it is impossible to apply treatments with substances suspected of leaving residues in honey (fig. 1) during the honey flow period which in most regions of central Europe between April and end of July. In order to reduce the *Varroa* population at this time of the year, only biotechnical measures can be used, such as the formation of nuclei and drone brood removal. These measures have to be integrated into beekeeping practice.



**Fig. 1: Treatment strategy**

A treatment strategy must consider the region, the development of the bee colonies, the varroa population dynamic, the periods of honey flow, the practice of beekeeping as well as the size of the apiary and the choice products applied. Before the beginning of the treatment the varroa population should not exceed 2000 mites. In August and September the population should be reduced with the help of treatments below 500 and in November below 50 mites.

In August and September, the Varroa population has to be reduced on time drastically by appropriate treatments, in order to keep the brood as little infested as possible, to enable a normal development of the winter bees. In November or in December, when the colonies are broodless, the Varroa population has to be reduced once more. With those two steps properly executed no supplementary treatment will be needed before the end of the honey harvest in summer of the following year. The substances used for Varroa control depend strongly on the climatic conditions, on the type of hive and the size of the apiary. For the hobby beekeeper the entire arsenal of efficient measures for alternative Varroa control is available. For professional exploitation, on the other hand, only few simple methods can be recommended. Some professional beekeepers have successfully carried out alternative control of *Varroa destructor* with almost thousand bee colonies.

## DYNAMIC OF THE VARROA POPULATION

Depending on the rearing period of the brood and on the re-invasions, a mite fall of several hundred mites per colony and year can be expected after treatment with Apistan<sup>57</sup>. Mossbeckhofer<sup>47</sup> has shown that the pyrethroid residues due to Apistan and Bayvarol treatments, present on the wax surface, continue their action even after the end of treatment. Thus, they prevent normal development of the Varroa population. This is the reason why it has been observed in the majority of apiaries treated with pyrethroids in the past, that the mite fall is 2 to 3 times smaller than in apiaries treated with alternative methods.

In a study carried out from 1989 to 1994 on a apiary containing approximately twenty colonies, the Varroa population was kept below the damage threshold by drone brood removal only (photo 1) and by formic acid treatments applied in two series of two to three short term applications each (photo 2, tab. 1). On this occasion, we observed that the more mites are over wintering, the more Varroa mites are suppressed by the drone brood removal in the following spring. With two or three times removing half a Dadant frame of drone brood we succeeded in suppressing up to 800 mites per colony. The average mite fall due to formic acid treatments was between 1000 and 2000 individuals.



*Photo 1: Drone brood removal  
The removal of drone brood helps to maintain the Varroa population at a low level.*



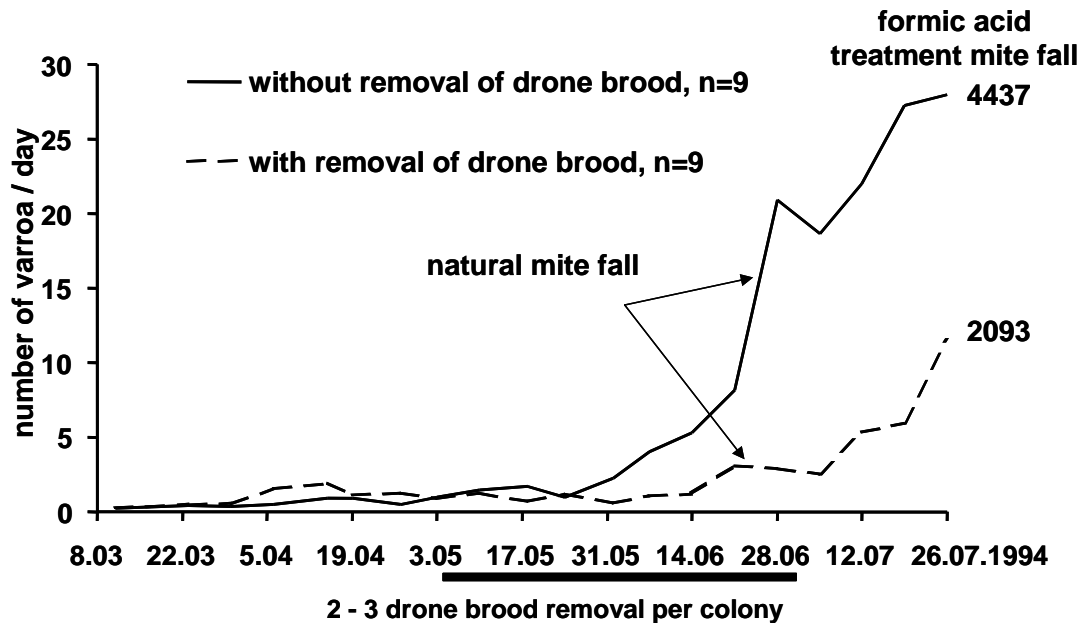
*Photo 2: Short term treatment with formic acid  
Two series of treatments, one in the beginning of August, the other at the end of September, each combining two to three applications within one week, eliminate 90 to 95% of the Varroa population.*

**Tab. 1: Drone brood removal and short term treatments with formic acid**

During six years we applied a control strategy combining two to three cuttings of drone brood with up to six selective formic acid treatments in August – September. The drone brood was washed, in order to determine the number of mites removed per colony. The natural mite fall and the one achieved by treatment were determined every week on mesh-protected inserts. At the apiary we installed two colonies treated continuously with Apistan, with the aim of determining the re-infestation rate. The great variability of mite fall due to treatments is caused partly by the varying quantities of drone brood removed and partly by the variations of treatment efficacy.

year	number of colonies	drone brood removal number of varroa removed			formic acid treatment mite fall			natural mite fall per day in October			re-invasion Varroa per colony n = 2
		av.	min.	max.	av.	min.	max.	av.	min.	max.	
1989	17	115	0	209	737	296	1714	0.03	0	0.08	398
1990	20	775	391	1404	6680	3125	10019	0.18	0	0.86	4250
1991	21	647	44	4602	1516	138	5501	0.09	0	0.36	154
1992	20	198	10	824	896	150	4119	0.21	0	1.25	172
1993	11	727	35	2090	1673	526	2719	0.10	0	0.29	217
1994	9	434	149	1229	2093	861	3707	0.27	0.05	0.50	213

The following question can be asked: How many over wintering mites can a colony tolerate in the frame of a strategy of alternative Varroa control? In the trial mentioned above no drone brood was removed in half of the colonies during the last two years of trial. Consequently, the mite fall due to formic acid treatment was two times higher in August and, with more than 4000 mites the level was too high<sup>18</sup> (fig. 2).



**Fig. 2: Drone brood removal**

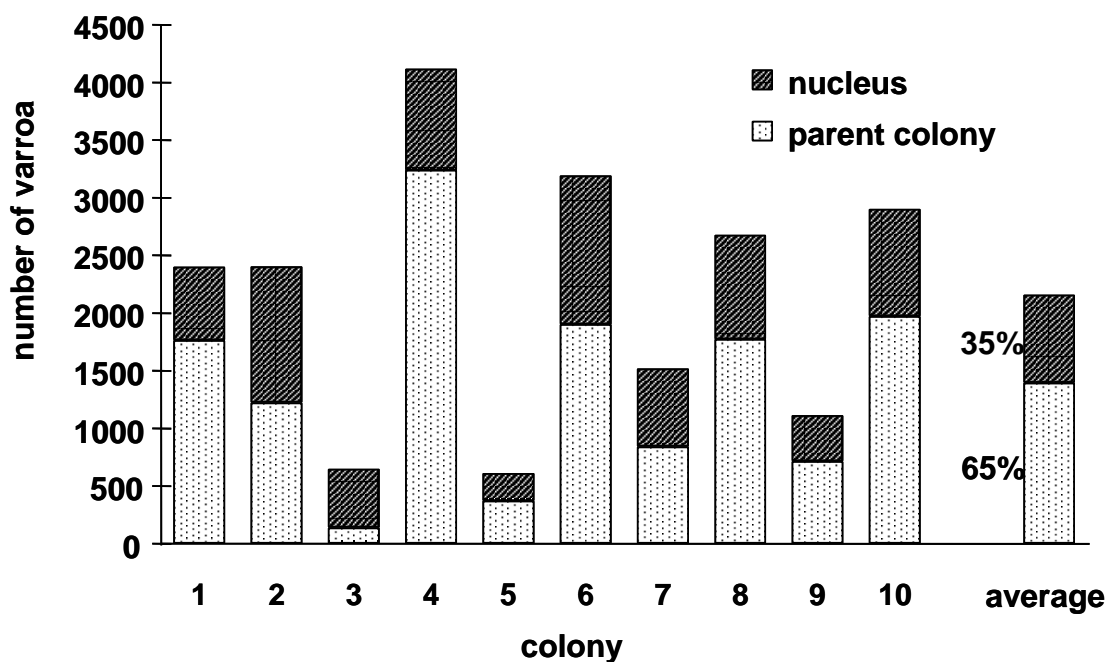
The formic acid treatment mite fall can be diminished by half by withdrawing the sealed drone brood of the drone brood comb.

The average natural mite fall in October was below 0.2 mites per day (tab.1). This corresponds to an overwintering population of about 100 to 200 mites. Imdorf and Kilchenmann (1990)<sup>34</sup> and Mossbeckhofer (2000)<sup>50</sup> proved a strong correlation ( $r > 0.8$ ) between the natural mite fall in October and the overwintering population. Therefore the natural mite fall of one Varroa per day corresponds to approximately 500 Varroa mites in a colony. This relation does not apply to treatments with thymol. In that case, one Varroa corresponds in the two weeks after the end of treatment to an remaining population of about 50 Varroa mites<sup>28</sup>.

In a control strategy, where a treatment mite fall of 1500 to 2000 mites is not exceeded, and if no drone brood removal is anticipated, the number of overwintering mites has to be reduced below 50 by means of a late treatment in the broodless colonies.

Re-invasions, such as in 1990 (tab. 1.), can significantly increase the mite population. In our study, we measured a re-invasion of over 4000 mites per colony during the whole year. These re-invasions were due to inadequate control of *Varroa destructor* in three neighbouring apiaries. The infested colonies perished from Varroaosis and were then pillaged by the trial colonies. During one week in August, a re-invasion of up to 2000 mites per colony was measured in two colonies treated continuously with Apistan. Therefore, control of the population by counting the natural mite fall is a good instrument against such bad surprises

The Varroa population in the mother colony is reduced by about one third by forming a nucleus containing about 50% of the sealed brood<sup>22</sup>. This is also reducing the later treatment mite fall a great deal (fig. 3). As the experience showed, an special treatment against the mites of the nucleus before August is not necessary.



**Fig. 3: Varroa population reduction in a mother colony by forming a nuclei**

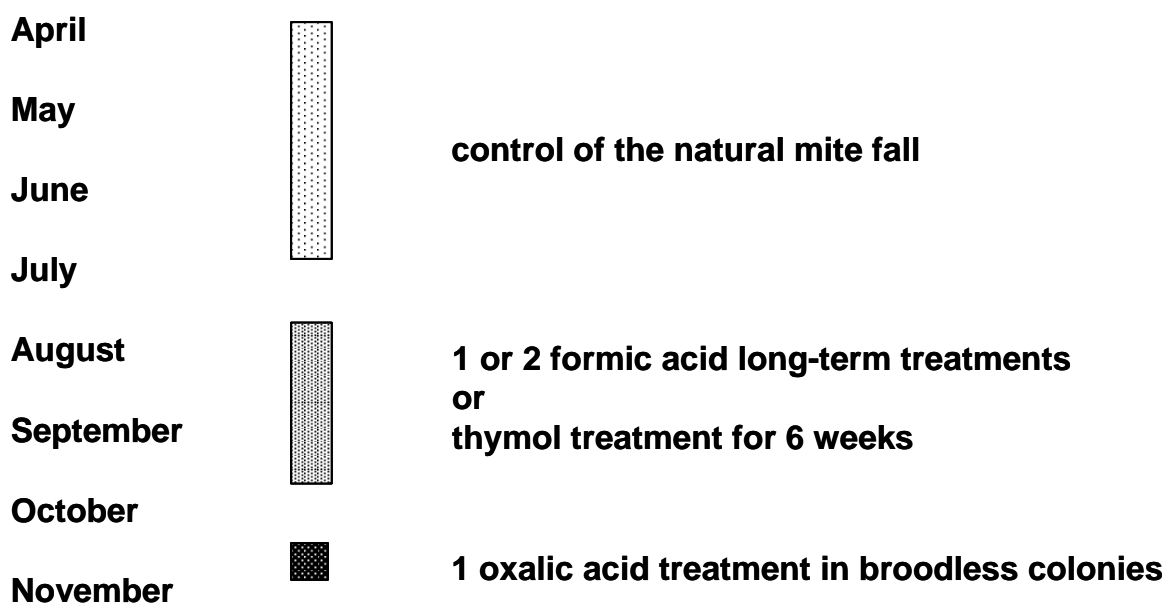
The formation of nuclei by withdrawing three Dadant frames with sealed brood reduces the Varroa population in the mother colony by about one third. In order to determine the Varroa population of the nuclei and the mother colonies, two successive Perizin treatments in the absence of brood were applied.

Hobby beekeepers can easily integrate these biotechnical measures in their control concept. For professionals, on the other hand, solutions have to be found with as little labour input as possible. Therefore, different European bee research institutes have been working during the last years for the improvement of different treatment methods. For example, short term formic acid treatments requiring a great deal of work were replaced by simpler long-term treatments. More than ten different devices for application have been developed for this type of treatment<sup>4,11,14,17,20,37,40,42,49,51,58</sup>. By the way, thymol application has been greatly improved and is currently the most used treatment in central Europe in the period from August to September.

The control strategy described below is used by a majority of beekeepers in different regions of central Europe. Beekeepers often apply variants of this strategy, which have not been tested and are therefore not safe.

## CONTROL STRATEGY

Regular surveillance of the Varroa population is an essential measure (fig. 4), to enable early detection of any increase in the parasite population and to induce the necessary control measures in time. After the honey harvest, in August and September the mite population has to be significantly reduced by applying one or two long-term treatments with formic acid or by a thymol treatment during 6 weeks approximately. As soon as the colonies are broodless, another treatment with oxalic acid must follow in November. If these measures are applied consequently, no supplementary treatment will be needed until the end of honey harvest in the following year.



**Fig. 4: Varroa control strategy for central Europe**

*The methods of alternative control of Varroa destructor are successful only, if integrated in a control strategy. The control of the degree of infestation, the reduction of the Varroa population in August and in September by formic acid or by thymol as well as the treatment with oxalic acid in November are the pillars of this strategy.*

## INFORMATION CONCERNING THE DIFFERENT INTERVENTIONS

### Surveillance of the Varroa population

If the number of resistant mites increases, the danger is quite real that the damage threshold will be sooner or later exceeded, and that colonies will perish. Massive re-invasions of neighbouring apiaries would be the consequence. It is necessary, therefore, to observe at specific times the Varroa population, from the beginning of spring until the end of July, by counting the natural mite fall by means of mesh-protected inserts (photo 3). In case of more than 30 mites counted per day, effective control measures have to be taken immediately, disregarding the period. The worst can be avoided by applying e.g. a long term formic acid treatment during one week. In table 2 the most important figures concerning the natural mite fall in the frame of this strategy are listed.



*Photo 3: Measuring of natural mite fall  
Mesh-protected inserts are indispensable devices in order to detect in time a strong degree of infestation, e.g. due to re-infestation or insufficient treatment efficacy. Thus, the necessary control measures can be carried out in time.*

**Tab. 2: Treatment strategy based on natural mite fall**

period	mites per day more than	measures to be taken
end of May	3	Perform a long-term treatment with formic acid immediately after the spring harvest.
end of July beginning of August	10	Two long-term treatments with formic acid are necessary.
whole season	30	The damage limit is exceeded. Immediate treatment is necessary.

### Reduction of the Varroa population in spring

If the presented strategy is applied consequently, no intermediary treatments - as described below - will be needed. However, if the treatments in autumn were not carried out correctly, or if re-invasions occurred, it is necessary to take measures rapidly. If more than three mites per day are counted by the end of May, it is advised not to wait until August, but to carry out one long term formic acid treatment for a week or two short term treatments, during the next period without honey flow. These treatments can increase the formic acid content in honey significantly <sup>6</sup>, for this reason they must be applied only in case of emergency.

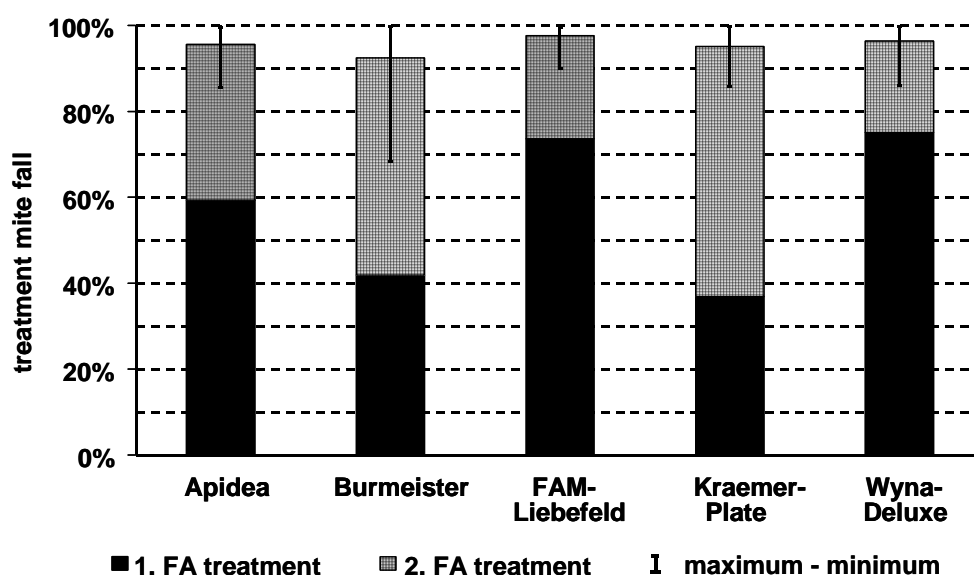
If the drone brood on the drone comb is removed two or three times, the Varroa population can be reduced by about half <sup>18,66</sup>, and by forming a nucleus by about one third <sup>22</sup>. The integration of these measures into beekeeping practice is still worth while, anyhow.

## Treatment against Varroa in August and in September

### Long-term treatment with formic acid

Various formic acid dispensers for long-term treatments are commercially available. In one type, a kind of absorbing material is soaked with formic acid<sup>20,36,49</sup> which evaporates through a surface with adjustable evaporation holes. In other dispensers, formic acid is contained within a reservoir and evaporates by means of a wick<sup>10,14,40,48,59,68</sup>. If the formic acid treatment (photo 4) is followed by an oxalic acid application, there is no need to reach the highest possible efficacy with formic acid. In this way, the risk of queen losses can be reduced. The instructions for use of the manufacturer should be followed with all dispensers.

The treatment efficacy depends on the ambient temperature, on the type of hive and on the colony strength. Various studies, e.g. that of Charrière et al. (1997)<sup>19</sup>, showed that a treatment efficacy of more than 90 % can be achieved by two long-term treatments (fig. 5.). Generally, the treatment efficacy is higher in hives with one brood chamber only than in those containing two chambers<sup>38</sup>.



**Fig. 5: Efficacy of different formic acid dispensers**

As shown in this trial, it is possible, in hives containing one brood chamber, to reduce the Varroa population by more than 90% with two formic acid long-term treatments. More than 10 different dispensers which exist on the European market show an overall good efficacy. In most cases, a slightly reduced efficacy in two-storey hives is to be expected.

### One or two treatments with formic acid?

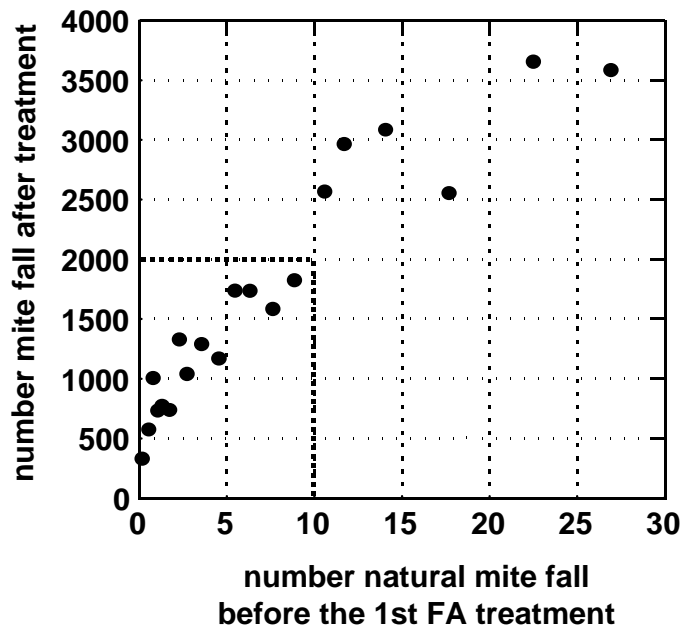
Depending on the degree of infestation, one or two long-term treatments (photo 4) with formic acid have to be carried out. If the number of mites exceeds 10 individuals per day in the beginning of August, two long-term treatments are needed. It is necessary to carry out the first treatment immediately after honey harvest and the second after the middle of September. If less than 10 mites per day are counted, a single treatment by the end of August will be sufficient.<sup>30</sup>. At that time a slightly better efficacy can be achieved compared to the beginning of August.



*Photo 4: Formic acid treatment with FAM-Dispenser*  
 One or two long-term treatments with a FAM-Dispenser during the period of August to September reduce the Varroa population by about 80 to 90%.

A treatment efficacy of 60 to 80 % can be expected with one treatment, depending on the climate and on the conditions of application. With two treatments, the efficacy increases to 90-98 %<sup>19,40,51,58,68</sup>. Note: formic acid affects also Varroa mites in their phase of reproduction within the sealed cells<sup>1,24</sup> and additionally it destroys the tracheal mite (*Acarapis*)<sup>25,41,65</sup>.

Why ten mites per day? The results in figure 6 show that a natural fall of 10 individuals per day corresponds to a treatment mite fall of about 2000 mites. If, in case of one single treatment, a treatment efficacy of 80 % is determined, about 400 mites remain in the colony. Trials showed that mite populations of this size present no risk for the health of colonies until the winter treatment. If, however, 3000 to 4000 mites are present in a colony, 1000 varroa per colony would still be counted after treatment, which are by far too many and a second treatment is therefore necessary.



**Fig. 6: The natural mite fall as an indicator for the varroa population size**

*In this trial, we compare the natural fall of Varroa mites in the beginning of August with the mortality of Varroa caused by subsequent treatments. Each point in this figure represents the average of ten colonies arranged in increasing order of the natural fall. These results show that, if the natural fall is below 10 mites per day, the mortality due to treatments will be below 2000 varroa. In this case, one single long-term treatment with formic acid, carried out at the end of August is sufficient to keep the brood which will give birth to winter bees on a low infestation level.*



### Treatment with components of essential oils

Thymol treatments can be carried out instead of formic acid treatments. Several products are commercially available. They contain thymol which is applied on a supporting material <sup>2,3,9,27,43,44,45,54,69</sup>. For the evaporation of the thymol, the products are placed on top of the brood combs during several weeks.

It is advised to follow the instructions for use of the manufacturer. After the honey harvest, the colonies have to be first of all fed abundantly. As with formic acid, the treatment has to be started as soon as possible, if the natural mite fall exceeds 10 mites per day. With products, where the tablets have to be replaced after 3 to 4 weeks, we recommend to finish feeding before using the second tablet (photo 5)



*Photo 5: Treatment with Apilife VAR  
Apilife-Var is a product on the basis of thymol which is used during 6 weeks in August and September. After a treatment for three a weeks the tablets are replaced by fresh ones. Depending on the type of hive and on the ambient temperature, the efficacy is above 90%.*

Under optimal conditions the treatment efficacy will vary between 90 and 98 % (tab. 3). No control of the treatment efficacy is needed, because in November, a supplementary oxalic acid treatment will be carried out.

**Tab. 3: Treatment efficacy of Apilife VAR**

*The product Apilife-VAR, containing thymol, has a very good efficacy under Swiss conditions in Swiss type hives (CH, combs perpendicular to entrance) and Ritter (R) hives, also a good efficacy was registered in Dadant hives (D, combs parallel to entrance).*

year	location	type of hive	number of colonies	efficacy of treatment with "Apilife VAR" per colony %	mite fall after treatment with "Apilife VAR"			Perizin control mite fall		
					average	min	max	average	min	max
1990	Säriswil A	CH	20	96.4	986	365	1704	37	3	168
	Hergiswil	CH	20	99.0	2453	917	4509	24	6	59
1991	Oeschberg	CH	12	93.7	277	19	859	18	0	64
	Grangeneuve	CH	12	95.6	1067	257	2355	55	5	143
	Säriswil A	CH	10	96.4	300	170	539	10	1	20
	Münsingen	CH	12	99.1	657	178	1338	5	1	13
	Hergiswil	CH	11	98.5	337	95	742	5	0	24
	Säriswil B	CH	11	98.2	151	87	284	3	0	9
	Salez	CH	9	98.7	223	67	431	3	0	15
	Bellechasse	R	16	96.7	713	469	1317	24	2	49
	Galmiz	D	19	91.7	986	465	1862	92	5	235

The different studies on thymol application <sup>27</sup> indicate that, for this method too, the treatment efficacy is – for a given dosage – increased in one-storey hives compared to two-storey hives (tab. 4). The dosage has to be adapted to the type of hive, to the volume of the hive and to the temperature. Probably, other products for the control of *Varroa destructor* will be available in future, containing components of essential oils.

**Tab. 4: Efficacy of thymol treatment in different types of hive**

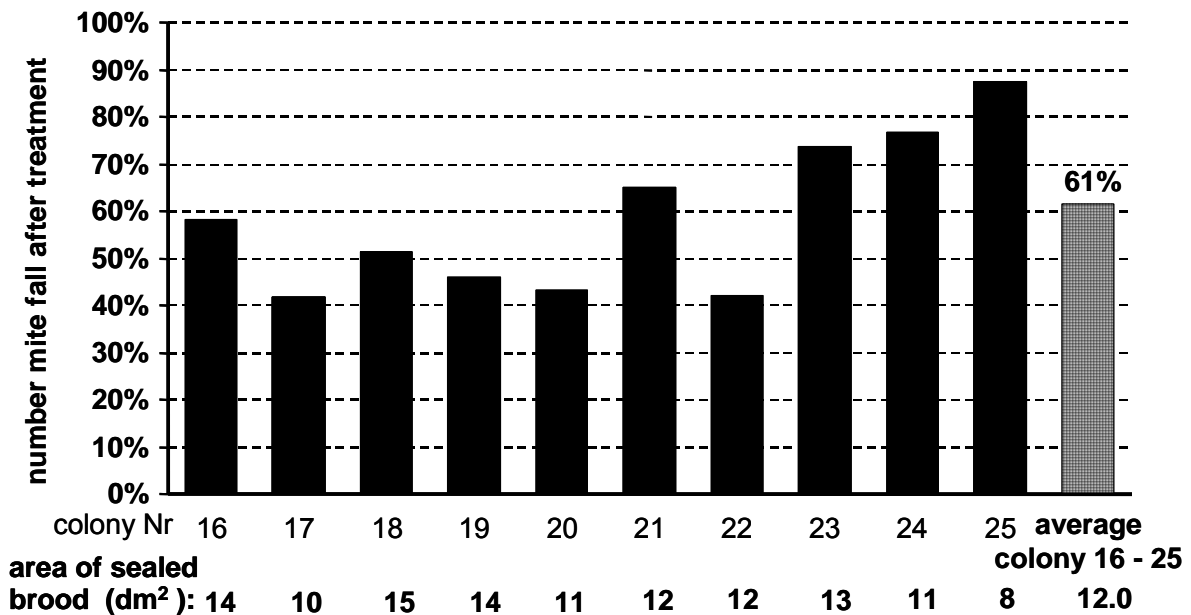
The results of different authors show that the only possibility to obtain a good efficacy with Apilife VAR is to adapt the dosage depending on the hive system used (one or two brood chambers) and on the temperature. Generally, the dosage, as well as the evaporation surface, have to be adapted. A treatment period of 4 to 6 weeks is sufficient. (More detailed information on the thymol application as well as the literature references of the given authors can be found in Imdorf et al. 1999<sup>27</sup>.)

authors	number of tablets	period of treatment days	number of brood chambers	type of hive	efficacy of thymol treatment average %
Rickli et al. 1991	2 x 1	38	1	CH	96.4
	2 x 1	79	1	CH	99.0
Mutinelli et al. 1991 1993	2 x 1	40	1	Dadant	89.0
	2 x 1	49	1	Dadant	68.7
Imdorf et al. 1994 1995	2 x 1	56	1	CH	97.7
	2 x 1	56	1	Dadant	91.7
Liebig 1993	2 x 1		1	Zander	97.4
	2 x 1		2	Zander	63.9
Schulz 1993	2 x 1		2	Zander	74.7
	2 x 2		2	Zander	94.9
	2 x 3		2	Zander	99.5
Calderone 1995 1999	2 x 2	19	2	Langstroth	96.7
	2 x 1	32	2	Langstroth	67.0

**Oxalic acid in broodless colonies**

This treatment in November is carried out, in order to reduce to a minimum the remaining Varroa population. If there is no re-invasion in spring, all control measures can be stopped until August of the following year. However, this objective can be reached only, if the colonies are broodless during the treatment, because oxalic acid has no effect on Varroa mites within the sealed brood.

Meanwhile, three different forms of application have been established: spraying, trickling and evaporation or more precisely sublimation<sup>12,15,33,39,52,53,55,60,61</sup>. All those applications have an efficacy rate of more than 95 % in broodless colonies (tab. 5, 6, 7, 8). In the majority of cases, the population of over wintering mites is reduced to less than 50 individuals (tab. 10, 11). If, on the other hand, sealed brood is still present, the efficacy rate drops strongly, depending on the brood surface. During a September treatment by spraying, of colonies with 12,5 dm<sup>2</sup> of sealed brood per colony, an efficacy rate of only 60 % was registered (fig. 7).



**Fig. 7: Efficacy of a treatment with oxalic acid by spraying applied to colonies with brood.**  
 In the beginning of September, the colonies sprayed with oxalic acid had on average of 12 dm<sup>2</sup> sealed brood.

**Application of oxalic acid by spraying**

For this application, a solution of 30 g oxalic acid dihydrate and one litre water is prepared and 3 to 4 ml of it are sprayed per frame side by means of an atomiser. Approximately 80 ml of this solution are needed for a strong colony, 65 ml for a colony of medium strength and 50 ml for a weak colony. This method is well tolerated by bees and is especially suitable for the treatment of colonies wintering in hives with one single brood chamber (e.g. Dadant) (photo 6).



*Photo 6: Spraying with oxalic acid*  
 Spraying with oxalic acid solution in autumn, as soon as the colonies are broodless, reduces the Varroa population below 50 mites.

This method is labour intensive (about 5 minutes per colony in single brood chamber) and is therefore convenient for hobby beekeepers with a small number of bee colonies. In our study, the average efficacy was about 97% and was independent of the user (tab. 5)<sup>33,60</sup>.

**Tab. 5: Efficacy of oxalic acid applied by spraying**

The average efficacy exceeds 95%. A Perizin control treatment was carried out three weeks after the oxalic acid treatment. The quantity of Varroa mites eliminated by these two treatments is considered as 100%.

year	apiary	hive system	number of colonies	treatment efficacy %			total number of varroa
				average	min.	max.	average
1994	Ins	DB	17	98.2	89.7	100	387
	Cormondrèche	DB	8	98.6	97.0	99.6	1007
	Spreitenbach	CH	17	98.8	85.7	100	95
	Zürich	CH	14	97.5	90.2	100	190
	Säriswil	CH	16	98.7	92.1	100	265
1995	Boden	DB	13	97.3	92.9	100	340
	Liebefeld	DB	14	94.5	76.9	100	724
	Wohlei	DB	14	97.6	92.0	99.3	733

**Application of oxalic acid by trickling**

For this type of application 35 g oxalic acid dihydrate are diluted in one litre sugar water 1+1. 5 ml of this solution are trickled into a bee way of bee-occupied combs. 50 ml of this solution are needed for a strong colony, 40 ml for a colony of medium strength and 30 ml for a weak colony. This method requires a relatively small amount of labour and is, therefore, convenient for exploitations with a great number of colonies.

The treatment efficacy in broodless colonies is in general more than 95 % (tab. 6.)<sup>12,16,39</sup> (photo 7). If one single application is applied in the indicated dosage, it will be very well tolerated by bees. No second treatment should be carried out at the end of autumn, especially in colder regions.



Photo 7: Trickling with oxalic acid  
The trickling method is the simplest application form of oxalic acid.

**Tab. 6: Efficacy of oxalic acid applied by trickling**

apiary	ml solution average	number of colonies	efficacy of treatment %			total number of varroa average
			average	min.	max.	
Boden	39	7	98.0	96.9	99.7	490
Hofen	45	8	97.7	87.6	99.6	430
Landikon	49	8	97.8	94.3	99.8	365
Pfeffikon	45	6	99.7	98.7	100.0	241
Schwand	41	6	97.8	94.8	99.5	503
Wohlei	46	6	98.3	96.6	99.7	1002
Zweisimmen	44	10	97.7	72.0	100.0	424

### ***Evaporation / sublimation of oxalic acid***

By evaporation of 1 g crystallised oxalic acid dihydrate in one-storey hives and of 2 g in Dadant and in two-storey hives, an efficacy of more than 95% was attained in broodless colonies (tab. 7, 8) <sup>63,64</sup>. An ambient temperature between 2 and 14° C during the treatment does not affect efficacy. In this method, the crystals are heated up to more than 200°C for two to three minute by means of an evaporator placed on the bottom of the hive (photo 8). The hive entrance is being closed by foam. Afterwards, the entrances of the hive remain closed during about ten minutes. All the bees, the hive walls and the combs are sprinkled with oxalic acid crystals. This method – well tolerated by bees – does not affect mites in sealed brood cells. This explains the lower efficacy of a treatment carried out, when sealed brood was present (tab. 8) <sup>63</sup>. At the moment, a great number of electric or gas evaporators are commercially sold. Not all of them have the same efficacy <sup>31</sup>. With this type of treatment the labour expenditure can be reduced by using several evaporation devices simultaneously.



*Photo 8: Evaporation with oxalic acid  
When evaporating oxalic acid, there is no need to open the colonies, and the treatments can be carried out at temperatures as low as 2° C.*

**Tab. 7: Dosage dependent efficacy of oxalic acid applied by evaporation**

In a trial carried out by Radetzki and co-workers (2000)<sup>64</sup> the optimum dosage for application of oxalic acid by evaporation was elucidated. By using one gram of oxalic acid dihydrate per colony, a high average efficacy is reached with a very small variation among various colonies. The trial showed that for good efficacy 2 g of oxalic acid should be preferably evaporated in hives with a big volume, such as Dadant hives or two-storey hives.

dosage of oxalic acid dihydrate (g/colony)	control un-treated	control water	0.5	1.0	2.0	3.0	5.0
	efficacy of oxalic acid treatment (%)						
average	1.2	4.8	82.8	96.0	97.2	99.0	99.2
minimum	0.0	1.3	51.7	93.2	91.7	98.1	99.0
maximum	2.7	11.5	96.0	99.2	99.6	99.7	99.8
number of colonies	6	8	12	10	13	11	5

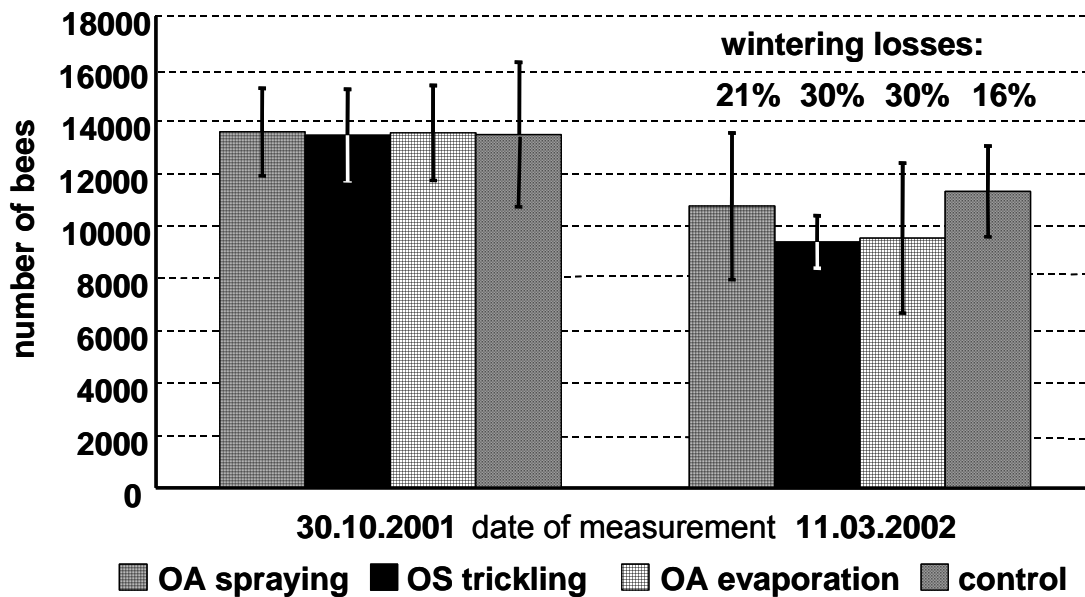
**Tab. 8: Efficacy of oxalic acid applied by evaporation**

In an extensive field trial, carried out by Radetzki and co-workers (2001)<sup>63</sup>, 1509 colonies were treated by evaporation of oxalic acid dihydrate in autumn 2000. The influence of the dosage, of the absence or the presence of brood as well as of the hive type on the efficacy against *Varroa* were evaluated.

factor	number of colonies	average efficacy
dosage		
2.8 g	723	94.8
1.4 g	474	94.9
no treatment	153	17.4
brood		
no sealed brood	535	95.9
with small amount of sealed brood	151	92.0
type of hive		
Dadant	165	92.0
1 brood chamber	150	95.4
2 brood chambers	531	94.8

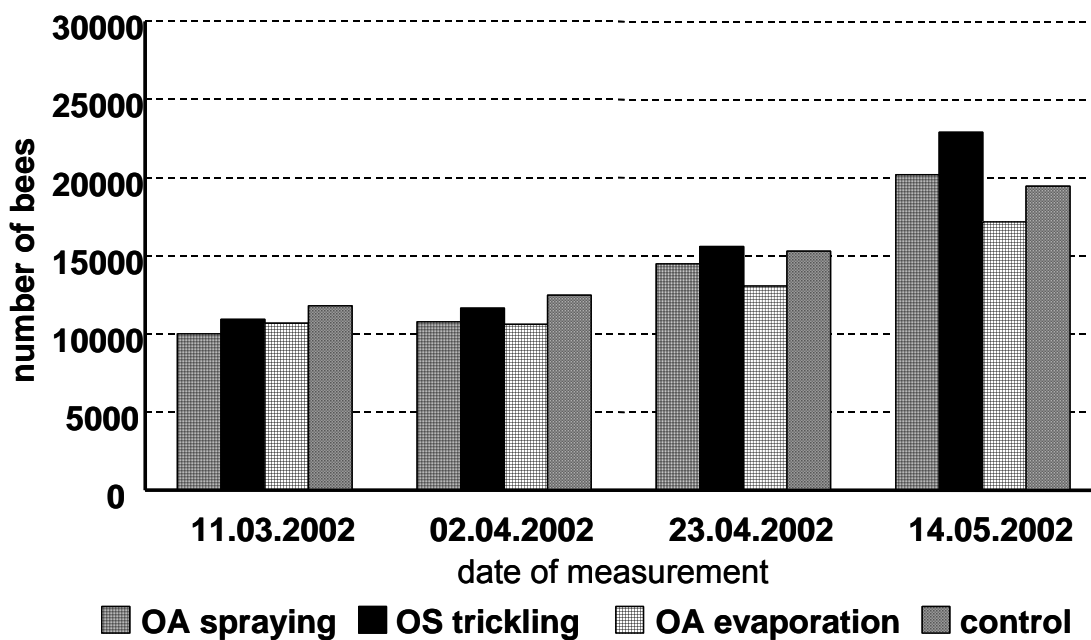
**Tolerance of oxalic acid by bees**

Recommended in the past in Italy<sup>53</sup>, the trickling of a solution of 60 g oxalic acid dihydrate per litre sugar water (1 + 1) was badly tolerated by bees, when applied under central European climate conditions. However, trials carried out in order to optimise the concentration showed, that 35 g oxalic acid dihydrate per litre sugar water were very well tolerated by bees, with the same efficacy of 95%<sup>16</sup>. Studies, carried out by different authors on the tolerance by bees of the three forms of oxalic acid application, indicate that there is no significant difference among them, compared to untreated control colonies. The bee losses during winter and the colony development in spring until the period of honey flow were determined (fig. 8,9)<sup>13,21</sup>. Summarising the results, all three methods are well tolerated by bees.



**Fig. 8: Bee tolerability of oxalic acid during winter time**

There is no significant difference between colony overwintering of the three groups treated with the different oxalic acid methods compared with the untreated control group. For this comparison, the number of bees was estimated in the end of October and in mid-March by the Liebefeld method<sup>29</sup>.



**Fig. 9: Influence of oxalic acid treatment on the colony development in spring**

The spring development of colonies is not affected by the form of application of oxalic acid.

## RESIDUES IN BEE PRODUCTS

### Formic acid and oxalic acid

Formic acid and oxalic acid occur naturally in honey<sup>23,35,53,56,70</sup>. A study over three years, where two long-term treatments with formic acid and one treatment with oxalic acid were carried out regularly, indicates that the formic acid content of spring honey is increased slightly to a level of 70 to

90 mg/kg of honey compared to untreated control honey, containing 30 to 45 mg/kg (tab. 9) <sup>6</sup>. From the toxicological point of view, this increase does not present a risk and cannot be detected by sensorial testing. In the succeeding honey harvests, no increase in the formic acid content was recorded in comparison to the natural content of honey <sup>8</sup>. If, however, formic acid is used in spring or in the beginning of summer - shortly before the honey flow - a strong raise of the formic acid content is to be expected in the following harvests, a raise which may be perceived even by tasting <sup>6</sup>.

The natural oxalic acid content of spring honey is not modified by a single treatment with oxalic acid (tab. 9.) <sup>5,6,53,62</sup>. In beeswax, there is no notable risk of residues, because the two acids are not fat soluble.

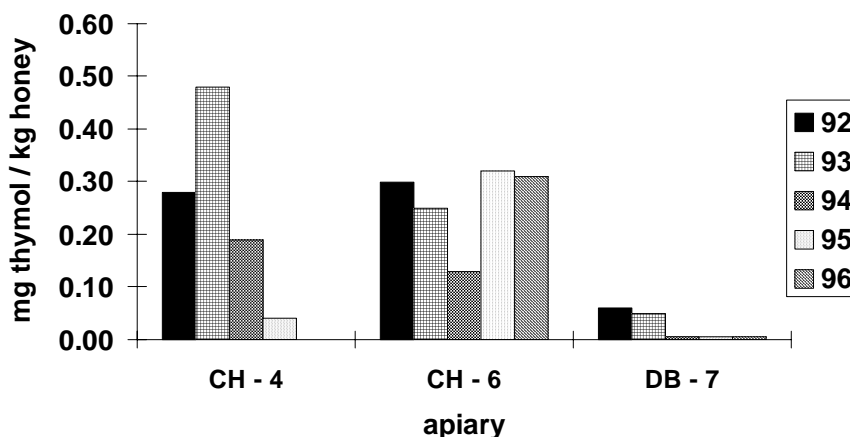
**Tab. 9: Residues of formic and oxalic acid in honey**

The average content of formic and oxalic acid in spring honey from ten apiaries, where alternative Varroa control with formic and oxalic acid was carried out three years, was analysed. This honey was compared to honey from control apiaries treated with Apistan only. This comparison allows to observe, whether the treatments cause an increase in the natural acid content.

year	1996		1997		1998	
	control	treatment	control	treatment	control	treatment
formic acid in honey mg/kg	45	94	31	91	41	71
oxalic acid in honey mg /kg	41	33	22	18	19	19

**Thymol**

Generally, the application of products on thymol basis leaves relatively important residues in beeswax (500-600 mg thymol per kg beeswax). However, it does not accumulate from one treatment to the next and it evaporates rapidly depending on the temperature <sup>7</sup>. After the treatment in August/September of the preceding year an average residue content of 0.1 to 0.2 mg thymol per kg honey are to be expected in the following spring harvest, depending on the type of hive <sup>7</sup> (fig. 10). From the toxicological point of view, these residues present no risk and do not alter the taste of honey. Only more than 1.1 mg thymol per kg honey is perceived by sensory testing <sup>8</sup>. This is why there is a Maximum Residue Limit of 0.8 mg thymol per kg honey in Switzerland.



**Fig. 10: Thymol residue in spring honey after thymol treatment in the previous year**



*In this trial conducted over 5 years, the colonies were treated with thymol annually at the end of the honey harvest during a period of 6 weeks. No residue accumulation of thymol in spring honey was found. The thymol content in honey from two apiaries with Swiss type hives (CH) was twice as much as that from an apiary with Dadant (DB) hives.*

## SAFETY MEASURES

For the beekeepers there is no health risk in treating the colonies with organic acids if they apply the safety measures described below, as the investigation of Gump et al. (2003)<sup>26</sup> has shown. When manipulating organic acids and essential oils, it is indispensable to wear acid resistant gloves. It is also recommended to wear goggles and to have a bucket filled with water ready at hand, when manipulating formic or oxalic acid. If a solution of oxalic acid is sprayed or if oxalic acid crystals are evaporated, a respiratory protection mask, type EN 149:2001 FFP2 or FFP3 must be worn for protection against acid aerosols in solid or liquid form. All the products designed for alternative Varroa control are being sold in specialised shops. The oxalic acid solution must be prepared by qualified personnel only.

## CONCLUSIONS

The application of these alternative control strategies<sup>32,67</sup> enables beekeepers in central Europe to keep the Varroa infestation beneath the damage threshold with reasonable additional labour (tab. 10, 11) and, at the same time, it assures high quality bee products.

### **Tab. 10: Efficacy of treatment combining formic with oxalic acid**

*The two objectives wished to be attained by this treatment strategy, to count less than 500 mites before the treatment with oxalic acid and less than 50 mites after treatment, have been attained. In one colony only, an over wintering Varroa population of slightly more than 50 individuals was observed. If a treatment efficacy of oxalic acid of 95% is estimated, the calculated over wintering population corresponds to the remaining 5%.*

apiary	number of colonies	efficacy of formic acid %			mite fall due to treatment with oxalic acid		calculated max. mite population in winter
		av.	max.	min.	av.	max.	
Baar	8	95	98	87	41	104	5
Boden	11	84	95	64	220	456	24
Frinvilier 96	9	95	99	88	174	416	22
Heitenried	14	95	100	69	47	155	8
Liebefeld	8	98	99	89	100	283	15
Salez	10	97	100	81	15	64	3
Schwand 96	7	97	99	86	36	68	4
Wohlei	11	97	100	92	52	157	8
Zweisimmen 96	9	73	91	45	135	291	15
Aenningen	20	96	99	95	72	86	5
Frinvilier 97	10	96	99	91	14	55	3
Grangeneuve	15	95	99	86	152	526	28
Landikon	8	89	97	75	85	269	14
Schwand 97	23	94	99	81	16	146	8
Zweisimmen 97	10	70	98	48	351	1147	60

**Tab. 11: Efficacy of treatment combining thymol (Thymovar) and oxalic acid**

In this treatment strategy, too, the two objectives of reducing the number of *Varroa* mites (see tab.10) are reached, in spite of unfavourable climate conditions.

apiary	altitude m	number of colonies	efficacy of Thymovar %			mite fall due to treatment with oxalic acid		calculated max. mite population in winter
			av.	max.	min.	av.	max.	
Varen	758	10	91	99	81	101	358	18
Moerel	762	8	87	94	76	131	260	14
Brig	880	10	77	91	40	191	733	39
Ritt	900	10	87	94	52	136	239	13
Ried	900	8	88	94	66	105	162	9
Rumeling	950	8	95	99	80	35	92	5
Birgisch	1090	6	63	82	35	144	255	13
St. Niklaus	1116	10	75	89	55	371	587	31
Ernen	1200	10	71	89	45	96	305	16
Buerchen	1335	10	82	89	71	253	488	26
Visperterminen	1367	10	75	80	68	392	541	28
Graechen	1611	10	95	97	90	91	184	9

As long as the pyrethroids were efficient, alternative *Varroa* control was carried out only by a small group of engaged beekeepers. However, since the mites have become resistant against pyrethroids, a majority of beekeepers have converted to alternative methods. Initially, by lack of experience, this conversion was accompanied by a great number of colony losses, comparable to those during the apparition of *Varroa destructor* in Europe. Facing Varroaosis, beekeepers cannot chose any longer. In order to successfully practice beekeeping, in the presence of resistant mites, the strategies of alternative *Varroa* control have to be carried out consistently, year after year.

The strategies of control developed for central Europe cannot be applied to other regions as such without change. Only consistent research over several years will yield optimum solutions for these regions.

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