FRAKNO THYMOL FRAME FOR CONTROLLING *VARROA JACOBSONI* –A FIELD TRIAL

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The "Thymol Frame" is recommended to beekeepers for combating Varroa mites. It is a device for thymol application in bee colonies. To date, available studies on this treatment were incomplete, so that a comprehensive evaluation of this method was not possible. The present study is intended to contribute towards widening knowledge on efficacy and residues in honey, with particular reference to the consequences of a treatment gap during honey flows.

THYMOL APPLICATIONS FOR COMBATTING VARROA

Treatment of bee colonies after the honey harvest

Application of Frakno Thymol Frame in a Swiss hive. In the upper frame, the drone brood is grown beneath the Thymol chamber.

Thymol application in bee colonies shows good effectiveness against *Varroa jacobsoni.* At the same time, it is well tolerated by the bees when the dosage is correct (Imdorf et al., 1995). In Switzerland, thymol is used mainly in the form of the registered products "Apilife VAR" (Imdorf et al., 1994) and "Thymovar" (Bollhalder, 1998). Treatment of colonies takes place after the end of the honey harvest over six to eight weeks from late summer until autumn. Under optimal conditions, efficacy is above 90%. This means that the Varroa population remaining in the colonies often exceeds the value desired for over-wintering. It is therefore recommended that the brood-less colonies should be given follow-up treatment, for instance with oxalic acid. When treatment is given after the harvest, thymol residues in honey lie far below the Maximum Residue Limit (MRL) of 0.08 mg thymol per kg honey (Bogdanov et al., 1998a). When treatment is repeated over several years, no excessive residues accumulate in honey or wax.

All year round treatment

For several years, another method, the so-called "Frank Thymol Frame" (Knobeispies, 1996) has been used in practice. In contrast to "Apilife VAR", this treatment is given throughout the year. Hereby 12g thymol crystals are placed into an evaporation box built into a brood frame and hung next to the brood nest. As soon as the thymol has evaporated, it is replaced about two to three times a year. Until now, there have been insufficient tests on efficacy and residue accumulation in honey.

In order to improve current knowledge in the two areas in question, the Bee Department, Liebefeld, carried out a study in co-operation with a group of beekeepers some of whom had used the thymol frame for several years. The intention was to document more extensively efficacy and thymol residues in honey. We would like to take this opportunity to thank these beekeepers for their valuable contribution.

First results about the contamination of honey with thymol were presented a year ago (Bogdanov et al., 1998b). Measurements were repeated during the second year of the trial in 1998. In addition, effectiveness against Varroa was monitored in several apiaries. Special attention was given to the question of whether interrupting the treatment during honey flows would contribute towards lowering thymol residues in honey. In this communication, the results are presented and an evaluation is made to establish under which conditions the thymol frame can be recommended for alternative Varroa control.

STUDY OF THYMOL RESIDUES IN HONEY

Method

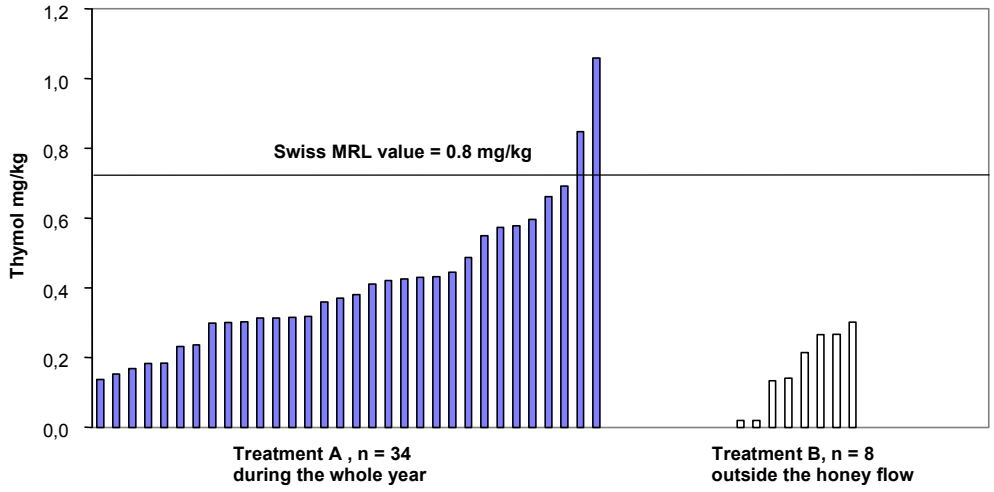
Altogether, the honey samples originated from the apiaries of 21 beekeepers. Two types of treatments were carried out: In type A, the thymol frames remained suspended in the brood box of the colonies throughout the year (long-term treatment). This variation was carried out by 17 beekeepers. In type B, thymol frames were removed at the beginning of April for the duration of the honey flow (4 beekeepers). During filling of the honey from the honey tank, containing the whole honey harvest, one sample was taken at the beginning, middle, and end of the filling procedure. Each sample was analyzed. For evaluation, an average of the three samples per apiary was considered. Since some participating beekeepers had hives in different locations, or had more than one honey harvest, there were a total of 34 honey samples from type A and 8 samples from type B treatment. The origin of the honeys was determined by electric conductivity measurements. Accordingly, 14 samples were blossom, 11 honeydew, and 9 mixed honeys from blossom and honeydew harvests.

Thymol contents were measured by gas chromatography (Bogdanov et al., 1998a). Limit of detection for thymol was 0.02 mg/kg.

Comparison of residues from long-term treatment and from interrupted treatment during honey flow

In Fig. 1, thymol residues in honey for all bottlings for both test variants are compared. When treatment is interrupted during honey flow (type B), significantly lower thymol residues occur in honey (Table 1) than with the long-term treatment (type A).

Fig.1. Thymol residues after treatment with thymol frame, honey crop 1998. Each column



represents an average of 3 samples, taken from each hive.

Table 1: Comparison of residue values in honey after long-term treatment (A) or after treatment outside honey flows (B), 1998

Treatment variant	Number of samples	Median value mg/kg	Minimum mg/kg	Maximum mg/kg	Standard deviation mg/kg
A	34	0.40	0.11	1.06	0.21
B	8	0.17	≤ 0.02	0.27	0.11

Residues with long-term treatment over two consecutive years

Residues in honey samples from the harvests of 1997 and 1998 were compared in 10 apiaries where thymol long-term treatment had been given since 1996 (Fig, 2). The average of all 10 apiaries for the second year of application 1997 was 0.40 thymol per kg honey and for year three 1998, 0.43 mg/kg. The differences between both years are low and statistically not significant. A general increase of residue values was thus not established.

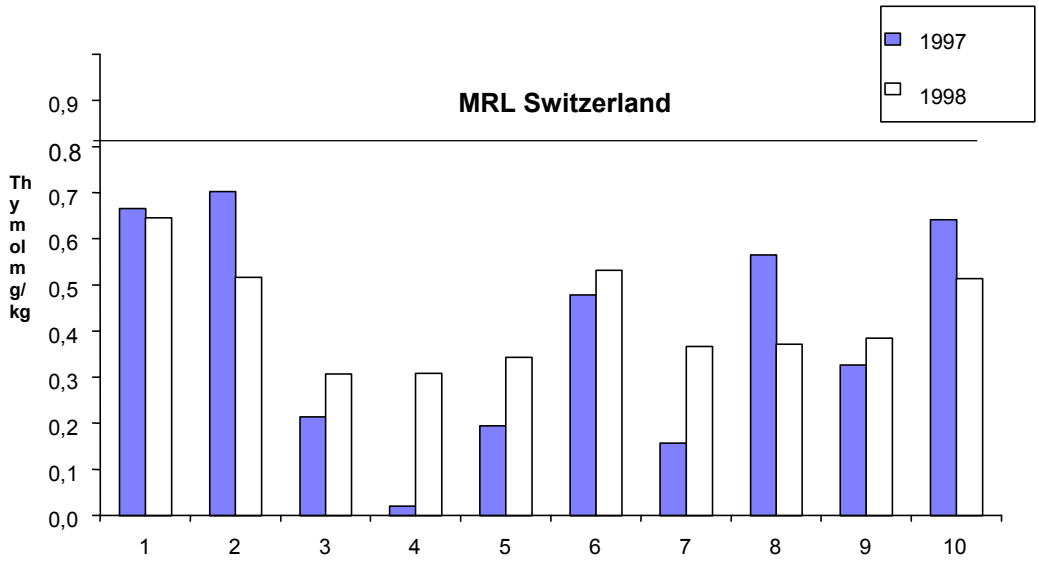


Figure 2. Residues in honey after long term treatment with thymol (variant A) after the second (1997) and third year (1998) of thymol use. The comparison was done in 10 apiaries.

Residues in relation to honey types

If different honey types are considered, there was a slight trend towards higher residue values in spring blossom honeys compared with the honeydew honeys of summer (Tab. 2). This difference is not statistically significant. In general, the amount of residue might also be influenced by the size of the honey harvest.

Table 2: Residues in different honey types after long-term treatment with the thymol frame

Honey type	year	number of samples	Average mg / kg	Minimum - Maximum mg/ kg
Blossom Honey	1997	6	0.52	0.40-0.83
	1998	14	0.46	0.13-1.06
Mixed Honey	1997	10	0.25	0.08-0.54
	1998	9	0.38	0.15-0.69
Forest Honey	1997	6	0.29	≤ 0.02-0.54
(honeydew)	1998	11	0.32	0.11-0.55

How are thymol residues to be judged in view of food law regulations?

Thymol is a natural constituent of some honeys, especially lime blossom honey. Maximum thymol concentration measured in lime blossom honey up to now is about 0.16 mg/kg (Table 3: Guyot et al., 1998). Amongst the honeys harvested in the present study there were no lime blossom honeys and only a few had a small proportion of lime harvest. The proportion of natural thymol in the samples analyzed can therefore be discounted. Average thymol contamination in honey after application of the thymol frame outside the

honey flow and of Apilife VAR is of the same magnitude of concentration as the measured maximum value of thymol in lime blossom honey. For long-term treatment with the thymol frame, however, values are 2-4 times higher (Table 3).

Table 3: Thymol residues in honey after different applications

Method of thymol application	Median value mg/ kg	Minimum-Maximum mg/kg
Thymol Frames 1997 Yearlong application in Switzerland (22 samples	0.33	≤ 0.02-0.83
Thymol frames 1997 (Wallner 1997) Year-long application in Germany (19 samples)	0.63	0.07-2.0
Thymol Frames 1998 Year-long application (34 samples)	0.40	0.11-1.06
Thymol Frames 1998 Application outside honey flow (10 samples)	0.17	≤ 0.02-0.32
Api Life VAR 8 weeks in autumn over 1-5 years (28 samples)	0.16	≤ 0.02-0.48
Lime blossom honey (Guyot et al. 1998)	0.08	0.02-0.16

All values found are safe, as regards health, but some may influence the taste of the honey, which runs counter to food law regulations. Food regulations in Switzerland, as well as those in the EU, prohibit additives to honey that alter its natural taste. The threshold concentration of thymol which alters honey taste, lies around 1.1 mg/kg (Bogdanov et al., 1998a). But persons with good sensory perception can also recognize lower thymol concentrations. Therefore thymol residues after thymol treatment must certainly lie below the Swiss MRL of 0.8 mg/kg. In the present study on long-term

treatment with the thymol frame, 5.4% of samples are above the tolerance value. In trials in Germany (Wallner, 1997), the Swiss MRL was exceeded by no less than 22% of samples. It can therefore be expected that the Swiss Cantonal chemists may query honeys from long-term treatment with the thymol frame.

EFFICACY OF THE FRAKNO-THYMOL FRAME AGAINST VARROA

Method for testing efficacy:

The success of the long-term treatment with the thymol frame was tested in 18 apiaries with 235 colonies by control treatment with oxalic acid spray, oxalic acid drip, or Perizin. The colonies were brood-less throughout. In this control treatment, over 90% of mites still present in the colonies fall on white, grid- protected inserts. Therefore the mite fall after control treatment corresponds nearly with the size of the mite population present in the colonies at wintering. It is known from other experiments that no more than 50 mites should over-winter. This value ensures that the mite population does not exceed the damage threshold until August of the following year. Exceptions are possible, especially when larger numbers of mites are carried in from outside (re-invasion).

Numbers of mites in the "Thymol frame colonies" before over-wintering

About two thirds of all colonies contained clearly more than 50 mites at wintering. In 16 of 18 apiaries there were colonies that contained more than 50 mites. In only 66 of 235 colonies the count of 50 mites was not exceeded. (Table 4).

How can this result be interpreted? - Assuming that the thymol frame long-term treatment is continued, significantly more than 50 mites can be tolerated. It is not possible to give the exact amount which colonies can tolerate well under these conditions due to lack of conclusive studies. An important consideration may be this: the last brood before wintering from which winter bees emerge, should not be parasitized excessively. In the present experiment a winter population of more than 250 mites was found in 42 of 235 colonies, and of more than 500 mites in 16 colonies. It can be assumed that a value of 500 over-wintering mites is at the upper limit that may be tolerated. In the present experiment this limit is exceeded by about 7% of colonies. This means that the success of the thymol frame long-term treatment cannot be graded as sufficient.

It cannot be estimated in how far re-invasions or propolized evaporation holes in the thymol frames played a part in the partially unsatisfactory effectiveness.

Table 4: Winter mite population after treatment with the thymol frame;

A=long-term treatment; B=treatment only outside honey flows

Apiary	Number of colonies	Mite fall of control treatment		
		Mean value	Minimum	Maximum
1 A	10	60	4	154
2 A	8	8	2	22
3 A	8	110	60	210
4 A	5	4	1	6
5 A	6	264	32	465
6 A	14	38	11	110
7 A	18	64	1	249
8 A	20	124	40	388
9 A	6	206	49	455
10 A	13	543	136	1284
11 A	21	165	42	650
12 A	15	310	85	1484
13 A	13	191	62	555
14 A	22	280	43	826
Mean value A	**179**	**181**		
15 B	8	55	8	365
16 B	16	161	38	426
17 B	18	148	17	342
18 B	14	77	44	151
Mean value B	**56**	**121**		

Influence of interrupted treatment during harvesting on efficacy

The shortened treatment outside harvesting had no negative effect on treatment success in this experiment (Table 4). An exact evaluation of effectiveness of this thymol application can only be made after a study over several years, where mite fall and colony development should be monitored weekly in a large number of colonies distributed in various locations.

CONCLUDING EVALUATION

In 5 of 18 apiaries there were some colonies which showed insufficient treatment success after long-term application of the thymol frame. Because this kind of treatment poses the additional danger that the harvested honey could contain too many thymol residues, the long-term treatment cannot be included in the Swiss Guidelines for Controlling Varroa jacobsoni. If the thymol frame is used outside harvesting and, depending an the size of the residual mite population, is combined with follow-up treatment in the brood less state at the end of October/beginning of November, this method may be considered as an alternative to other permitted thymol applications.

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