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# Residues in wax and honey after Apilife VAR® treatment

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**Abstract** – Apilife VAR®, with thymol as its main active ingredient, is registered for use against *Varroa jacobsoni* Oudemans in Switzerland. After Apilife VAR® treatment in autumn of 1992, the residues in honey and comb were examined the following spring. Only thymol residues were found in honey, whereas in comb the residues consisted of 99 % thymol and 1 % menthol. The thymol residues in honey did not increase with an increasing number of treatments and varied between 0.02 to 0.48 mg·kg$^{-1}$ with an average of 0.15 mg·kg$^{-1}$ ($n = 29$). The taste threshold of thymol in acacia and rape honey was between 1.1 and 1.6 mg·kg$^{-1}$. The brood comb in two apiaries, where Apilife VAR® was used for, on average, 4 consecutive years, had a mean content of 574 mg·kg$^{-1}$ and this did not increase with an increasing number of treatments. The thymol residues in honey comb were on average 21.6 mg·kg$^{-1}$. Thymol did not evaporate during comb melting, but decreased rapidly when comb and foundation were exposed to the air during storage. © Inra/DIB/AGIB/Elsevier, Paris

**honey / wax / residue / thymol / Apilife VAR®**

## 1. INTRODUCTION

The use of synthetic lipophilic acaricides leads to accumulation of these substances in beeswax and less so in honey [1, 17]. The accumulation in wax depends on the frequency, lipophilicity and amount of active ingredient used. The residues in wax are very persistent and the acaricide level drops very slowly after termination of treatment [1]. The persistence of pyrethroid acaricides in wax has led to resistance of *Varroa jacobsoni* against fluvalinate and flumethrine [11]. For that reason, different non-toxic and non-persistent acaricides such as organic acids, thymol, and essential oils can be used

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for mite control [8, and references therein]. The Italian product Apilife VAR® is an acaricide with thymol as its main active ingredient. It has been used for *V. jacobsoni* control in the past years in Italy [13], Switzerland [6, 9, 14] Germany [10] and Austria [12]. When treatments are made at the end of summer, the efficiency varies between 92 and 99 % [ 6]. In Switzerland, Apilife VAR® has been registered as a varroacide since 1996. In previous studies we dealt mainly with the optimization of Apilife VAR® use against *V. jacobsoni* [6, 9]. In the present work we report on the residues in honey and beeswax after long-term use of Apilife VAR®.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Apilife VAR®, used for *Varroa jacobsoni* treatment and supplied by LAIF Chemicals, Vignonza, Italy contains approximately 20 g of a mixture of thymol (76 %) , eucalyptol (16.4 %), menthol (3.8 %) and camphor (3.8 %), in a pad of florist block material. All reagents were of analytical grade. The solvents used were of analytical grade for residue analysis.

### 2.2. Methods

#### *2.2.1. Apilife VAR, treatments and sampling*

Two applications, each of one Apilife VAR® pad, were applied beginning in August 1992 after the honey harvest for a total duration of 6–8 weeks. The treatments were carried out in six apiaries with Swiss hives (CH) and two apiaries with Dadant (D) hives.

Sugar feed samples: about 10 $cm^2$ of wax from each comb of the hive containing sugar-feed were scratched down to the foundation with a spoon. The sugar-feed and the comb were separated by sieving.

Comb samples: about 10 $cm^2$ of wax from each brood and honey comb in the hive that did not contain sugar-feed or honey were scratched down to the foundation with a spoon just after the treatment in autumn or in April of the next year.

Honey samples of mixed floral origin were harvested in the period between May and June of the following year after the Apilife VAR® treatment. Thymol is a natural aromatic component of lime honey; however, there was no significant contribution of lime nectar in the examined honeys, as tested by pollen and sensorial analysis.

New wax samples: old brood comb without brood, sampled in April, was melted into new wax at the test apiaries by a vapour wax melter (Bourgeois, BJL 36).

#### *2.2.2. Evaporation from wax*

Fifty grams thymol was placed on the bottom of a cardboard box containing brood comb and foundation in the upper part. This box was placed in a thermostat-controlled incubator at 30 °C and after 1 month all of the thymol had evaporated. The comb and the foundation were then stored under the following conditions:

1) ten sheets of foundation, stacked in a closed cardboard box;

2) ten frames of brood combs, placed in a Dadant hive brood chamber without bees, with a grid-protected top and bottom, exposed to the air from above and below;

3) ten sheets of foundation, mounted on comb frames exposed to air;

4) ten sheets of foundation, placed in a super of a Swiss type hive occupied by bees.

Storage experiments numbers 1, 2 and 3 began on 17 July 1995 and were conducted for 50 weeks in a unheated room at ambient temperature to imitate storage in a Swiss bee house. Experiment 4 began on 15 May 1995 and continued for 6 weeks. Samples for GC analysis were taken at different times from each of the ten sheets of foundation or frames of comb, and were frozen and ground with a coffee mill while still frozen to yield a single sample for each sampling time. In experiments 1 and 2 a small piece from each foundation (1–2 g) was cut out with a knife. In experiment 4, the samples were taken by cutting out a piece of the comb together with the foundation. The comb samples from experiment 3 were taken with a spoon as in the field experiments (see above).

#### *2.2.3. Diffusion of thymol from wax into honey*

Wax was melted with different amounts of thymol to reach the following thymol con-

centrations: 20, 50, 100, 200, 500, 1 000 and 2 000 mg·kg$^{-1}$. Thirty grams of each wax sample were poured into Petri dishes (three dishes per thymol concentration) to build a wax layer. Thirty grams of acacia honey were then poured into each Petri dish. After storing the Petri dishes at 30 °C for 30 days the honey was carefully poured out and analysed.

### 2.2.4. Sample extraction and GC analysis

Before extraction all samples were kept in a freezer.

Comb and wax: the samples were homogenized by grinding them while frozen with a coffee mill. One gram of ground sample was dissolved in 10 mL ethanol in a glass centrifugation tube (Sorvall Art. 00771) and sonified for 1 h in an ultrasonic bath (Bandelin Sonorex TK52). The ethanol extract was separated from the wax particles by centrifugation for 20 min in a Sorvall RC5C centrifuge, equipped with a SM-24 rotor at RCF-maximum of 22 800 at 4 °C. The supernatant was transferred to a new centrifugation tube. The remaining wax components in the supernatant were then removed by repeated freezing, subsequent centrifugation and decantation of the clear supernatant as described above. Five millilitres of the clear supernatant were added to 20 mL of water, ready for SPE. Six mL, 500 mg SPE columns (Baker Art. 7020–06), mounted on a Supelco SPE manifold (Art. 5-7 030), were activated with one column volume of ethanol and one volume of water. The diluted wax extract was then passed through the SPE column. The substances were eluted with 2 mL acetone and a spatula of $Na_2SO_4$ was added to absorb the water. This extract was kept in auto-sampler vials in the freezer until GC analysis.

Honey and feed: 10 g honey or sugar feed were dissolved in 20 mL of 20 % (v/v) aqueous ethanol, passed through the activated SPE columns (see above), eluted with 2 mL acetone and further treated in the same way as the wax eluate (see above). One millilitre of extract was injected with an autosampler in splitless mode (1 min) on a 30 m DB-5 MS capillary column, 0.32 mm ID, film thickness 1 mm (J + W), mounted in a Hewlett Packard 5 890 chromatograph, equipped with an FID detector and $H_2$ carrier gas. The temperature program was as follows:

honey and feed extracts: 1 min. 50 °C, 2 °C/min until 130 °C, 10 °C/min until 220 °C, 30 °C/min until 280 °C, 60 min;

wax and combs extracts: 1 min 50 °C, 4 °C/min until 180 °C, 30 °C/min until 280 °C, 60 min.

The response of the FID detector was linear in the whole determination range and no dilutions of extracts were necessary. The concentrations in honey and wax were calculated with the external standard method, as shown by the following examples:

honey and feed: concentration (c) in sample in mg·kg$^{-1}$ = (c) in extract (mg·L$^{-1}$)/ 5;

wax, comb: concentration ( c ) in sample in mg·kg$^{-1}$ = (c) in extract (mg·L$^{-1}$) × 5;

where 5 is a dilution (wax, comb) or a concentration (honey, feed) factor.

The external standards of all reference substances (eucalyptol, Fluka 46 090; menthol (–), Fluka 63 660; D (–) camphor, Merck 9656; and thymol, Fluka 89 330; all delivered with analytical certificates) were made up in blank extracts: 0.5 mg·L$^{-1}$ honey or feed extract and 1 mg·L$^{-1}$ comb or wax extract (see chromatograms on *figure 1*).

In preliminary experiments, the recoveries of all substances from honey and sugar-feed, both containing about 95–99 % sugar, were tested and were found to be the same. Also, about 50 different floral honeys of Swiss origin, without Apilife VAR® pre-treatment, were tested and no peaks with the retention time of eucalyptol, camphor, menthol and thymol could be detected. The validation results for thymol, shown in *table I*, were carried out with a mixed floral honey. In trace analysis, recoveries lower than 70 % are generally corrected. The thymol recoveries below 0.5 mg·kg$^{-1}$ were between 61 und 67 % and were corrected for 100 % recovery by multiplying the raw values by 1.59. The recoveries of the other substances in honey and combs were similar, but were not tabulated, as these substances were not important for the present study. No other peaks, other than thymol, could be found in honey and only trace levels of menthol were found in comb (see also Results). The recoveries, shown in *table I*, were determined in the whole determination range with comb samples, because comb was the main matrix analysed in this study. However, the recoveries of all substances in comb and wax (main matrix is wax) were found to be equal. In comb samples only eucalyptol could not be analysed with the present method. For this substance a different capillary column (DB-210, J + W) was used and only trace amounts (about 0.4 mg·kg$^{-1}$) were found, even when the thymol concentration exceeded 1 000 mg·kg$^{-1}$. The limit of detec-

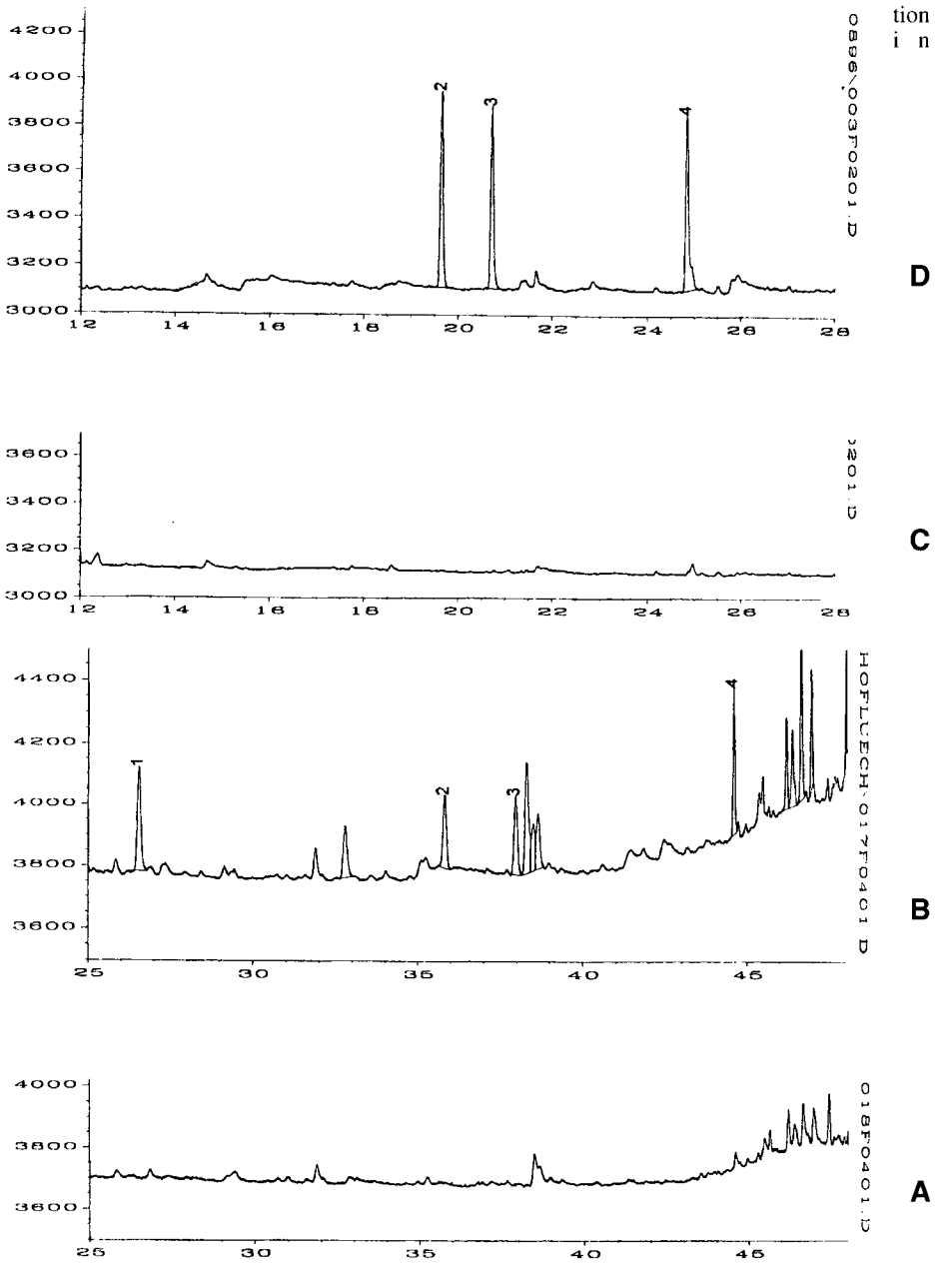


**Figure 1.** Chromatogram profiles of honey and brood comb extracts. **A:** Blank honey extract. **B:** Blank honey extract containing 0.5 mg·L$^{-1}$ of eucalyptol (1), camphor (2), menthol (3) and thymol (4). **C:** Blank brood comb extract. **D:** Blank brood comb extract, containing 1 mg·L$^{-1}$ of camphor (2), menthol (3) and thymol (4).

**Table I.** Recoveries of thymol in honey and combs. Honeys and combs were spiked with different amounts of thymol. Six repetitions were made for each concentration level. The value are averages ± standard deviation.

| mg·kg$^{-1}$ | Honey recovery % | rel.sd % | mg·kg$^{-1}$ | Comb recovery % | rel.sd % |
|---|---|---|---|---|---|
| 0.10 | 66.6 | 10.0 | 2 | 86.5 | 8.8 |
| 0.25 | 60.6 | 4.1 | 10 | 82.2 | 7.5 |
| 0.50 | 61.2 | 8.3 | 100 | 99.3 | 6.8 |
| 0.75 | 87.5 | 6.0 | 500 | 93.9 | 7.5 |
| 1.00 | 93.5 | 9.9 | 2 000 | 96.4 | 6.0 |
| 2.00 | 94.1 | 7.7 | 4 000 | 91.5 | 8.1 |
| 10.00 | 94.8 | 6.8 | | | |

honey was 0.01 mg·kg$^{-1}$ for eucalyptol and 0.02 mg·kg$^{-1}$ for the other substances. In wax it was 0.4 mg·kg$^{-1}$ for all substances.

The long-term recoveries were controlled by analysis of reference honey and wax samples. The reference honey and wax samples contained 0.5 mg·kg$^{-1}$ (honey) and 10 mg·kg$^{-1}$ (wax) of each Apilife VAR® component. In a series of ten samples, one reference honey or wax sample was analysed. The recoveries for each substance were controlled by a control chart [15]. In all cases, the recoveries of the Apilife VAR® components in the reference samples varied within the 2 sigma limit of the control chart. When honey, spiked with 5 mg·kg$^{-1}$ camphor, menthol and thymol was stored in closed jars for 2 years, no evaporation of these substances could be detected as the contents of all substances remained constant during this period.

All analyses were carried out under the conditions of quality assurance (QA) according to the EN 45001 norm in order to ensure long-term reproducibility of the measurements.

### *2.2.5. Sensory tests*

Acacia and rape honey, containing different amounts of thymol, were examined by the Institute's sensory panel, which is accredited according to the EN 45 001 norm. The taste threshold of thymol in honey is the lowest concentration, the taste of which was found to be significantly different from that of the same honey without thymol. The tasting method used was a paired comparison test between honey samples containing thymol and control samples (with no thymol) according to the DIN Norm 1977. The significance level $P$ was 0.05.

## 3. RESULTS

No eucalyptol, camphor and menthol were found in all examined honey samples. In wax, besides thymol, only small quantities of menthol (< 1 % of the thymol residues) were detected. Thus we report only on thymol residues.

### 3.1. Field tests

In the first experiment, the thymol residues in brood wax and feed were examined in eight apiaries just after treatment and in the following spring (*table II*). There was considerable variation in the residue level from apiary to apiary. Immediately after treatment with Apilife VAR® in autumn, the residues in the brood comb and in the feed were relatively high with averages of 2 121.5 and 4.6 mg·kg$^{-1}$, respectively, but then decreased by factors of 2.3 in the feed and 3.2 in the brood combs the following spring. The average thymol residue level in spring sugar feed was 2 mg·kg$^{-1}$, which is 13 times higher than the average residues found in honey (see below). In two apiaries treated with Apilife VAR® for 4 consecutive years, the residues in brood comb wax did not increase with an increasing number of Apilife VAR® treatments, and were on average 574 mg·kg$^{-1}$ (minimum 58.9 and maximum 1 989 mg·kg$^{-1}$,

**Table II** Residues of thymol in feed and brood comb wax in autumn 1992 and spring 1993 after treatment with Apilife VAR®, in August 1992.

| Apiary | Feed mg·kg$^{-1}$ | | Brood comb wax mg·kg$^{-1}$ | |
|---|---|---|---|---|
| | Autumn 92 | Spring 93 | Autumn 92 | Spring 93 |
| 1-CH | 6.19 | 3.63 | 2 176.9 | 560.3 |
| 2-CH | 5.45 | 1.06 | 662.6 | 446.5 |
| 3-CH | 2.07 | 0.68 | 3 035.8 | 578.2 |
| 4-CH | 3.94 | 1.26 | 2 149.0 | 788.1 |
| 5-CH | 7.54 | 1.48 | 1 692.8 | 387.9 |
| 6-CH | 4.71 | 3.28 | 4 753.9 | 1 988.6 |
| 7-D | 4.72 | 2.79 | 1 632.0 | 168.6 |
| 8-D | 2.15 | 1.73 | 868.8 | 432.0 |
| Average | 4.60 | 1.99 | 2 121.5 | 668.8 |

Abbreviations: CH: Swiss hive, D: Dadant hive.

*figure 2A*). The thymol residues in the brood combs of the Dadant hives, with an average of 399 mg·kg$^{-1}$, were considerably lower than those of the Swiss hives which had an average of 966 mg·kg$^{-1}$. When the treatment was stopped in one apiary in 1993, the residues dropped in the following year to a level of almost zero (*figure 2A*).

The thymol residues in brood and honey combs and in foundation, made by melting old brood comb, were followed for three consecutive years in three different apiaries (*figure 2B*). There was no increase in residues with increasing years of treatment. The thymol residues in the honey combs with an average value of 21.6 mg·kg$^{-1}$ were much lower than those in the brood combs of the same apiaries with an average content of 516.8 mg·kg$^{-1}$. Also, the residues in brood comb and in the foundation made out of this brood comb did not differ significantly. This demonstrates that there is no evaporation of thymol during the melting process (see procedure in method section).

In honeys from eight different apiaries, treated for 2–5 consecutive years, there was no increase in thymol residues with increasing number of Apilife VAR® treatments (*figure 2C*). The average thymol content was 0.15 mg·kg$^{-1}$, with a minimum of 0.02 and a maximum of 0.48 mg·kg$^{-1}$ ($n$ = 29). The thymol residue level in honeys from the Swiss hives with an average of 0.19 mg·kg$^{-1}$ was higher than that in the Dadant hives with 0.05 mg·kg$^{-1}$. After termination of treatment in 1993, in apiaries 1 to 5, a dramatic decrease in the residue level was observed in the honey of the following year. In three of the apiaries the residue level in the honey of 1994 dropped below the detection limit of 0.02 mg·kg$^{-1}$. In another two apiaries the residues in 1994 dropped by 53 % to reach the detection limit of 0.02 mg·kg$^{-1}$ in 1995.

### 3.2. Evaporation from wax

*Figure 3* shows the thymol evaporation from wax under different conditions. When thymol-spiked combs were stored in a Dadant hive brood chamber without bees (experiment 2), the thymol evaporation rate was lower, and a final concentration level equal to about 40 % of the initial value was reached after 1 year. Only about 30 % of the initial thymol evaporated from foundation stacked in a closed cardboard box after 1 year of storage (experiment 1). However, if foundation was stored in frames exposed

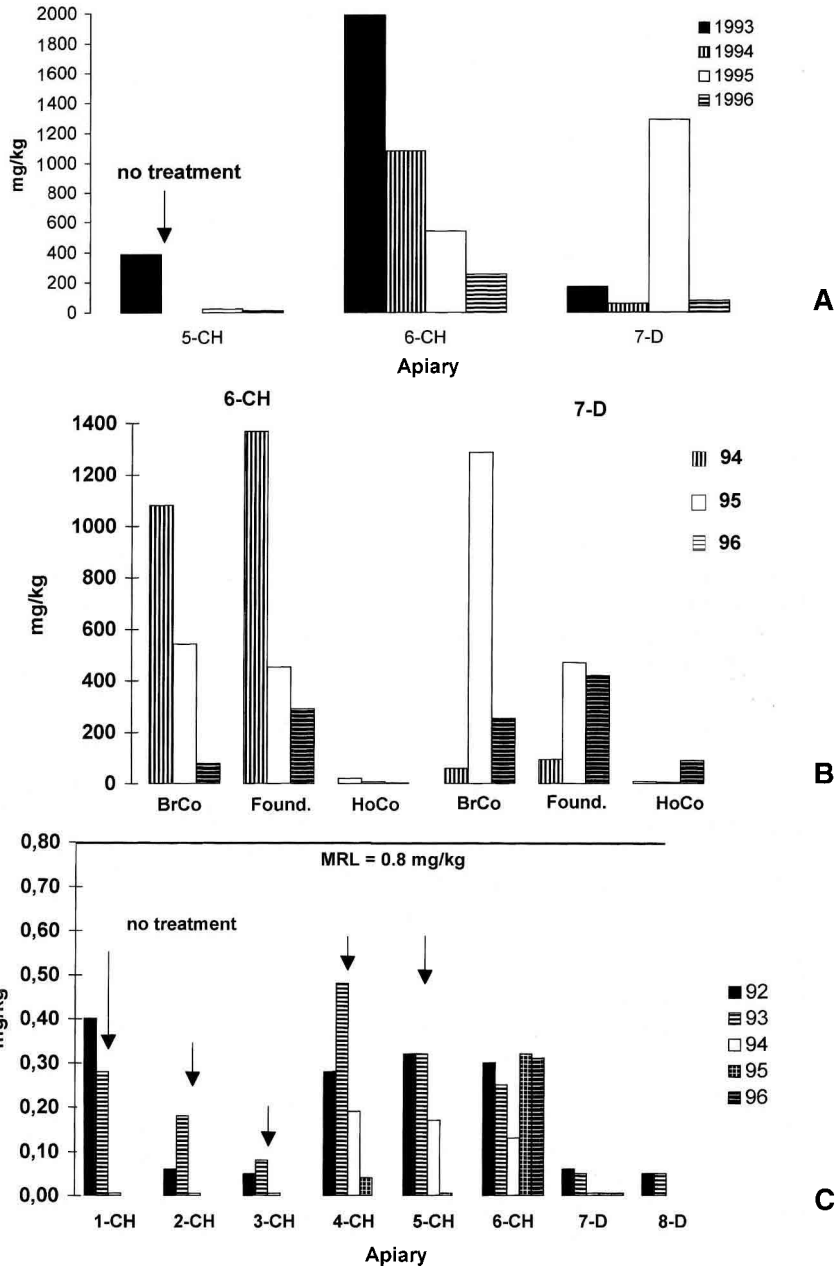


**Figure 2.** Thymol residues after field trials with Apilife VAR®. **A:** Brood comb wax: in apiary 5-CH no treatment was made in 1993, treatment termination is indicated with an arrow. **B:** Brood combs (BrCo), foundations (Found.) made out of the brood combs and honey combs (HoCo) in two apiaries: CH: Swiss hives, D: Dadant hives. **C:** Thymol in honey. In apiaries 1- to 5-CH no treatments were made in 1993: treatment terminations are indicated with arrows. MRL: Maximal residue limit. For further details, see Methods.

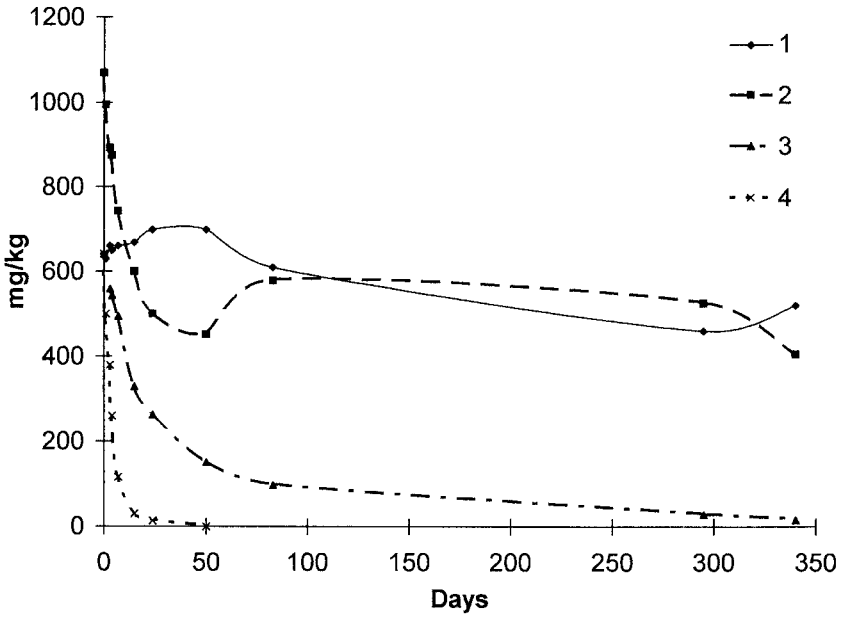


**Figure 3.** Evaporation of thymol under different conditions. **1:** Foundations, stacked in a closed cardboard box. **2:** Brood combs, placed in a Dadant hive brood chamber unit without bees. **3:** Foundations, mounted on comb frames exposed to air. **4:** Foundations, placed in the super of a bee-occupied Swiss type hive

to air (experiment 3), thymol evaporated rapidly and reached a zero level after 1 year. When foundation was placed in a Swiss beehive in May during the honey flow, the evaporation was extremely rapid and the thymol concentrations in the combs reached zero level within 2 weeks (experiment 4).

### 3.3. Diffusion of thymol from wax into honey

Thymol diffuses into honey out of thymol-spiked wax in a linear, concentration-dependent manner (*table III*). The correlation coefficient of a linear regression of honey thymol concentration versus wax thymol level was 0.997. The Swiss maximal residue limit (MRL) for honey of 0.8 $mg \cdot kg^{-1}$ was reached when the thymol wax concentration was about 800 $mg \cdot kg^{-1}$.

### 3.4. Influence of thymol on honey taste

The taste threshold concentration of thymol in acacia and rape honey was between 1.1 and 1.6 $mg \cdot kg^{-1}$. When the thymol concentrations reached and exceeded the taste threshold, the test participants did not show a significant preference for control honey over thymol-spiked honey.

## 4. DISCUSSION

After application of Apilife VAR® there were considerable residues of thymol in wax and feed in the brood comb, but only small residues in honey. The residue level in both honey and wax did not increase with an increasing number of Apilife VAR® applications. This is most probably due to the volatility of the product: thymol wax

**Table III.** Diffusion of thymol from beeswax into honey in Petri dishes, coated with wax containing different amounts of thymol.

| Thymol mg·kg$^{-1}$ in wax | 20 | 50 | 100 | 200 | 500 | 1 000 | 2 000 |
|---|---|---|---|---|---|---|---|
| Thymol mg·kg$^{-1}$ in honey | ± 0.02 | 0.06 ± 0.04 | 0.06 ± 0.04 | 0.22 ± 0.04 | 0.44 ± 0.08 | 1.09 ± 0.07 | 1.90 ± 0.06 |

30 g acacia honey was stored in the dish for 2 months at 30 °C. The values are averages of a triplicate ± standard deviation.

residues decrease extremely rapidly in the hive and to a lesser degree when stored in a location with good airflow.

We did not detect adverse effects on the bee populations and on the bee behaviour of the treated hives that could be attributed to the high thymol wax levels. Lower wax residues of the other Apilife VAR® ingredients, menthol and camphor, should be expected after *V. jacobsoni* treatments because these are more volatile than thymol. In in vitro experiments, camphor and menthol showed strong *V. jacobsoni* toxicity together with a good tolerance by bees [6], but they have not been tested in field trials.

Assuming a similar diffusion from honey combs into honey as that of our model diffusion experiments, the theoretical thymol level in honey, which originates from diffusion of comb thymol into honey, can be determined. In this case one would expect a thymol honey concentration at the detection level of 0.02 mg·kg$^{-1}$ in the honey from two apiaries that had an average honey comb thymol content of 21.6 m·kg$^{-1}$ (*figure 1B*). However, the average thymol found in the honey of these two apiaries was 0.16 mg·kg$^{-1}$. The thymol level in honey of the apiary with Swiss hives was 0.29 mg·kg$^{-1}$, which is much higher than the one of the apiary with Dadant hives with ≤ 0.02. mg·kg$^{-1}$. We presume that the major part of the thymol honey residues originated from the feed. Indeed, the thymol feed level in spring with an average of 2 mg·kg$^{-1}$ was very high, but was similar in the Swiss and in the Dadant hives (*table II*). The difference in the honey thymol level in Swiss and Dadant hives can be explained only by the general observation in Switzerland that in spring there is always much more sugar-feed in Swiss hives than in Dadant hives. Indeed, all high residue values were found in Swiss hives, while honey from Dadant hives had residues around the detection limit (*figure 1C*). In order to keep the thymol residue level at a minimum, we recommend that combs used during Apilife VAR® treatment should not be used as honey combs in the following year and that brood combs with feed should not be present during honey flow.

The highest concentration found after Apilife VAR® application was 0.48 mg·kg$^{-1}$, which is considerably lower than the thymol taste threshold in honey, which is between 1.1 and 1.6 mg·kg$^{-1}$. For safety reasons the Swiss MRL (maximum residue limit) was fixed at 0.8 mg·kg$^{-1}$. After Apilife VAR® treatments in Germany [16] the average honey thymol level was 0.12 mg·kg$^{-1}$, while in Italy values between 0.1 and 0.3 mg·kg$^{-1}$ were measured [13]. Thymol is a natural aromatic component of lime honey and values in the range of 0.02 to 0.16 mg·kg$^{-1}$ have been measured [5]. Thus, the maximum thymol concentration measured in lime honey corresponds to the average concentration found in honeys after Apilife VAR® application. Thymol, as well as eucalyptol, camphor and menthol, have an FAO GRAS status (generally recognised as safe) in concentrations up to 50 mg·kg$^{-1}$. According to EU regulation No. 2377/90

thymol is in group II of the non-toxic veterinary drugs, which do not need an MRL.

However, if thymol is used during a honey flow, there is considerable danger that thymol residues in honey might reach levels above the taste threshold. In Germany a *V. jacobsoni* treatment method is promoted that uses thymol-filled frames during the whole year. In honeys harvested in bee hives treated with this method, the thymol residues were in some cases higher than the MRL value of 0.8 mg·kg$^{-1}$ [2, 18]. Therefore, Apilife VAR® or thymol treatments should only be made in autumn after the honey harvest.

**Résumé – Résidus dans la cire et le miel après un traitement à l'Apilife VAR®.** L'Apilife VAR®, dont le thymol constitue la principale matière active, est un varroacide efficace, enregistré depuis 1996 en Suisse dans la lutte contre l'acarien *Varroa jacobsoni*. On a examiné les résidus d'Apilife VAR® dans le miel et la cire au printemps qui a suivi le traitement, effectué en août durant 6 à 8 semaines à la dose de deux tablettes. Le camphre, le menthol et le thymol ont été isolés de la cire et des rayons par extraction à l'éthanol, purification par congélation, centrifugation des débris de cire et extraction de la phase solide (SPE). L'eucalyptol, le camphre, le menthol et le thymol ont été isolés dans le sucre de nourrissement et le miel par SPE. Tous les composés des extraits de miel et de cire ont été analysés par chromatographie gazeuse sur capillaires avec détection FID. Les taux de recouvrement du thymol ont varié entre 80 et 100 % dans la cire et entre 60 et 95 % dans le miel (*tableau I*). Seuls des résidus de thymol ont été trouvés dans le miel, alors que dans les rayons et la cire les résidus étaient constitués à 99 % de thymol et 1 % de menthol. Juste après le traitement, les résidus de thymol dans les rayons à couvain et dans le sucre de nourrissement étaient relativement élevés avec des moyennes de 2 121,5 et 4,6 mg·kg$^{-1}$, respectivement. Mais le printemps suivant, ils étaient 2,3 fois moins élevés dans la nourriture et 3,2 fois dans les rayons à couvain (*tableau II*). Les rayons à couvain dans deux ruchers où l'Apilife VAR® avait été utilisé en moyenne durant quatre années consécutives, avaient une teneur moyenne en thymol de 574 mg·kg$^{-1}$ et ce chiffre n'a pas augmenté avec le nombre de traitements (*figure 2A*). Les résidus de thymol dans les rayons à couvain et à miel et dans la cire gaufrée, obtenue par fusion des vieux rayons, ont été suivis durant trois années consécutives dans trois ruchers différents traités à l'Apilife VAR® (*figure 2B*). On n'a pas observé d'augmentation des résidus de thymol avec les années. Dans les rayons à miel, leur valeur moyenne, de 21,6 mg·kg$^{-1}$, était bien inférieure à celle des résidus dans les rayons à couvain (516,8 mg·kg$^{-1}$). Les résidus de thymol dans les rayons à couvain et dans la cire gaufrée issue de ces rayons ne différaient pas significativement. Cela prouve que le thymol ne s'évapore pas au cours de la fonte des vieux rayons. Le thymol diffuse de la cire dans le miel selon un mode linéaire lié à la concentration (*tableau III*). La limite maximale de résidu en Suisse (MRL) est de 0,8 mg de thymol·kg$^{-1}$ de miel, valeur qui peut être atteinte lorsque la concentration du thymol dans la cire avoisine 800 mg·kg$^{-1}$. Les résidus de thymol dans le miel n'ont pas augmenté avec le nombre de traitements et ont varié entre 0,02 et 0,48 mg·kg$^{-1}$ (moyenne 0,15 mg·kg$^{-1}$, N = 29, *figure 2C*). La seuil de détection par le goût du thymol dans le miel d'acacia et de colza s'est situé entre 1,1 et 1,6 mg·kg$^{-1}$. Une valeur de MRL de 0,8 mg·kg$^{-1}$ a été établie pour la Suisse sur la base des analyses chimiques et sensorielles. Le thymol est un composant naturel du miel de tilleul dans des concentrations allant jusqu'à 0,16 mg·kg$^{-1}$. Il possède un statut GRAS (reconnu généralement comme sain) pour des concentrations qui vont jusqu'à 50 mg·kg$^{-1}$. Un traitement à l'Apilife VAR® au mois d'août n'affecte donc pas négativement la qualité des miels. On a étudié l'évaporation du thymol dans différentes

conditions (*figure 3*). Lorsque les rayons étaient stockés dans un entrepôt à ruches aéré, 60 % environ du thymol initial s'évaporait. Si les cires gaufrées étaient stockées à température ambiante avec une circulation libre de l'air, l'évaporation était très rapide et le niveau zéro était atteint au bout d'un an de stockage. Si elles étaient placées dans les hausses d'une ruche suisse pour être étirées par les abeilles, le thymol s'évaporait complètement en deux semaines.


**miel / cire / résidu / thymol / Apilife VAR®**

**Zusammenfassung – Rückstände in Wachs und Honig nach Anwendung von Apilife VAR®.** Apilife VAR® mit dem Hauptwirkstoff Thymol ist ein wirksames Akarizid, das in der Schweiz seit 1996 für die Varroabekämpfung registriert ist. Nach Behandlungen im August mit 2 Tafeln Apilife VAR während 6–8 Wochen, wurden im darauffolgenden Frühjahr die Rückstände in Honig und Wachs untersucht. Die Extraktion von Campher, Menthol und Thymol aus Waben und Wachs wurde mit Ethanol durchgeführt, die anschliessende Reinigung erfolgte durch Ausfrieren, Zentrifugation und SPE (Solid Phase Extraktion). Eucalyptol, Campher, Menthol und Thymol wurden durch SPE aus Zuckerfutter und Honig isoliert. Alle Substanzen wurden mittels Kapillargaschromatographie mit FID Detektion bestimmt. Im Honig wurden ausschliesslich Thymolrückstände gefunden. Im Wachs bestanden die Rückstände aus 99 % Thymol und 1 % Menthol. Im Wachs lag die Thymolausbeute zwischen 80 und 100 %, im Honig zwischen 60 und 95 % (*Tabelle I*). Unmittelbar nach der Behandlung waren die Rückstände in den Brutwaben und im Futter mit Durchschnittswerten von je 2121.5 und 4.6 mg·kg$^{-1}$ relativ hoch. Bis zum nächsten Frühjahr nahmen sie jedoch um den Faktor 2.3 im Futter und 3.2 in den Brutwaben ab (*Tabelle II*). Auf zwei Ständen, die mit Apilife VAR® behandelt wurden, lagen die Brutwabenrückstände im Durchschnitt bei 574 mg·kg$^{-1}$ und erhöhten sich nicht mit zunehmender Anzahl der Behandlungen (*Abb. 2A*). Die Thymolrückstände in Brutwaben, Honigwaben und Mittelwänden, die aus Wachs von eingeschmolzenen alten Brutwaben produziert waren, wurden auf 3 verschiedenen Ständen für die Dauer von 3 Behandlungsjahren verfolgt (*Abb. 2B*). Die Thymolrückstände erhöhten sich nicht mit zunehmender Anzahl der Behandlungen. Die Rückstände in den Honigwaben, mit einem Durchschnitt von 21.6 mg·kg$^{-1}$, waren viel kleiner als diejenigen in den Brutwaben, die einen Durchschnitt von 516.8 mg·kg$^{-1}$ aufwiesen. Die Thymolrückstände in Brutwaben und in den Mittelwänden, die aus diesen Waben produziert wurden, waren gleich. Das bedeutet, daß Thymol während des Schmelzens der Altwaben nicht verdampft. Thymol diffundiert aus Wachs in den Honig in linearer Abhängigkeit der Thymolwachskonzentration (*Tabelle III*). Der schweizerische Toleranzwert für Honig von 0.8 mg·kg$^{-1}$ wurde bei einer Thymolwachskonzentration von ca. 800 mg·kg$^{-1}$g erreicht. Die Thymolrückstände im Honig erhöhten sich nicht mit zunehmender Anzahl der Behandlungen und variierten zwischen 0.02 und 0.48 mg·kg$^{-1}$, mit einem Durchschnitt von 0.15 mg·kg$^{-1}$ ($n$ = 29, *figure 2C*). Der Geschmacksschwellenwert von Thymol in Akazien- und Rapshonig lag zwischen 1.1 und 1.6 mg·kg$^{-1}$. Der Toleranzwert für Thymol wurde auf Grund der Rückstandsanalysen und der sensorischen Tests auf 0.8 mg·kg$^{-1}$ festgelegt. In Lindenblütenhonig gibt es natürlicherweise bis zu 0.16 mg Thymol·kg$^{-1}$. Thymol hat einen GRAS (Generally Recognised As Safe) Status. Das bedeutet, daβ bis zu 50 mg Thymol pro kg Honig gesundheitlich unbedenklich sind. Zusammenfassend kann man sagen, daβ bei Anwendung von Apilife VAR® im August die Honigqualität nicht beeinflusst wird. Die Verdampfung des Thymols aus thymolhaltigem Wachs wurde unter verschiedenen Bedingungen untersucht. Wenn Waben in einem Bienenkasten

ohne Bienen gelagert werden, verdunsten ungefähr 60 % des Thymols. Wenn Mittelwände belüftet werden und bei Zimmertemperatur gelagert werden, verdunstet Thymol relativ schnell und verschwindet aus dem Wachs nach einer Lagerungsdauer von einem Jahr (*Abb. 3*). Werden Mittelwände in einem Aufsatz eines Schweizer Kastens im Frühling ausgebaut, verdunstet das Thymol vollständig innerhalb 2 Wochen. 

**Honig / Wachs / Rückstände / Thymol / Apilife VAR®**

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