# *Varroa* control preceding honey flow; thymol and formic acid residue

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The ectoparasitic mite *Varroa destructor* is a pest of the honey bee *Apis mellifera*. The mite can, among others, be controlled by evaporation of formic acid or thymol in the bee hive. In this study we have determined thymol and formic acid residues in honey in case a honey super is placed on a hive immediately after termination of a *Varroa* control with formic acid or thymol (Thymovar) in early spring. The study was conducted with three groups of 10 honeybee colonies placed on a location where a good honey flow could be expected. Two groups were treated with Thymovar and formic acid respectively. The third group was not treated and used as a control. The thymol and formic acid residues in the honey exceeded significantly the thymol and formic acid residues in the honey from the control group. However, the thymol concentration was always below the taste threshold and the formic acid concentration was most of the time below the taste threshold.

*Keywords: Varroa* control, formic acid, thymol, residue, honey, Thymovar

The ectoparasitic mite *Varroa destructor* is a pest of the honey bee *Apis mellifera*. In the Netherlands *Varroa* control with a combination of a biotechnical method plus organic acids or Thymovar is recommended. Thymovar and formic acid are advised to be applied at the end of the summer after honey harvest. In case of a heavy *Varroa* infestation, a spring treatment is advised.

In this study thymol and formic acid residues in the honey are determined in case a honey super is placed on a hive, immediately after termination of a treatment with Thymovar or formic acid in early spring.

## MATERIALS AND METHODS

Thirty queen right honeybee colonies in 10-frame hives (Spaarkasten) were placed in an area were a good honey flow could be expected. Each honeybee colony was equipped with a *Varroa* bottom board (Universalboden Theodor

Martin). The 30 honeybee colonies were at random divided into three groups of

10 honeybee colonies. The 10 honeybee colonies of each group were placed next to each other. The distance between the three groups was 5 m at least. One group was treated with formic acid and one group was treated with Thymovar. The third group was used as the control group and was not treated. To ensure that the growth of the honeybee colonies was not impeded, an extra brood chamber was placed under the honeybee colonies.

Treatments

Thymovar was applied according to the instructions that are provided with the commercial product. The treatment with Thymovar was started on 19 March 2003 (day 0). On each honeybee colony one Thymovar plate was applied on top of the combs. On day 21 removal of the plates from the honeybee colonies ended the treatment with Thymovar. Thymol evaporation from the Thymovar plates was not determined.

The treatment with formic acid was started one week after the start of the Thymovar treatment (day 7). For this treatment a Nassenheider evaporator was used with 60% formic acid. The formic acid treatment ended on day 21. Evaporation of the formic acid was determined after 4, 7 and 14 days. The recommended evaporation rate is 6-10 ml per day.

Honey harvesting

On day 21, directly after the treatments were ended, honey supers were placed on every honeybee colony. Three weeks later the honey supers were taken off. The honey was harvested directly after the removal of the honey supers. The sealed honey was sampled in glass sampling jars. After harvesting the samples were stored at 5°C for two weeks.

Analysis of the honey

The honey samples from the honeybee colonies that were treated with formic acid were analysed spectrophotometrically. The analyses were carried out with a Boehringer Mannheim testkit. The honey samples from the control honeybee colonies were analysed in the same way.

The honey samples from the honeybee colonies that were treated with Thymovar were analysed by de Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek, Zeist (TNO). This analysis was performed with GC-MS. The honey samples from the control honeybee colonies were analysed in the same way.

Statistical analysis

Formic acid residues and thymol residues were compared between the treatment and the control using ANOVA ($P < 0.05$).

*Table 1.* Effect of a formic acid treatment before honey flow on residues in the honey (mean ± standard deviation, range in brackets).

| Treatment | Number of honey bee colonies | Formic acid residue (mg/kg)* |
|---|---|---|
| control | 10 | 48.6a ± 17.9 (28.7-91.6) |
| formic acid | 10 | 143.6b ± 43.5 (71.8-205.3)** |
| taste threshold | | between 150 and 600 mg/kg |

*numbers followed by a different letter are significantly different; **4 colonies had a formic acid concentration above 150 mg/kg

## RESULTS AND DISCUSSION

### Formic acid

There was a significant difference between formic acid residue levels in the honey of the colonies that were treated with formic acid (average 143.6 mg/kg) and the formic acid residue levels in the honey from colonies that were used as controls (average 48.6 mg/kg, Table 1). Due to the formic acid treatment the formic acid residue levels in the honey had increased. As there is no MRL (Maximum Residue Level) for formic acid, the taste threshold was used as a maximum. The average formic acid residue level in the honey from honeybee colonies that were treated with formic acid just remained below the taste threshold which is between 150 mg/kg and 600 mg/kg (Bogdanov *et al.* 1999). The highest formic acid residue that was measured was 205.3 mg/kg, which exceeds the lower limit of the taste threshold. This means that in some cases the formic acid in the honey can be tasted.

There was a significant variation between the honeybee colonies that were treated with formic acid, in the amount of evaporated formic acid (minimum 145 ml, maximum 280 ml). In some cases the amount that evaporated exceeded the recommended amount. The formic acid residue found in the honey also showed a significant variation (minimum 71.8 mg/kg, maximum 205.3 mg/kg). However, there was no correlation between the two. A high evaporation resulted in both low and high formic acid residue levels. The same goes for a low evaporation.

Formic acid is a natural component of honey. Variation in formic acid residue levels between the honeybee colonies that were not treated was significant. This variation in natural formic acid residue was also determined by Bogdanov *et al.* (2002).

### Thymovar

There was a significant difference between the thymol residue levels in the honey of the honeybee colonies that were treated with Thymovar (average 0.384 mg/kg) and the thymol levels in the honey from honeybee colonies that were used as controls (average 0.036 mg/kg, Table 2). Due to the Thymovar treatment

*Table 2*. Effect of a Thymovar treatment before honey flow on residues in the honey (mean ± standard deviation, range in brackets).

| Treatment | Number of honey bee colonies | Thymol residue (mg/kg)* |
|---|---|---|
| control | 10 | 0.036a ± 0.011 (0.020-0.060) |
| Thymovar | 10 | 0.384b ± 0.120 (0.270-0.600) |
| taste threshold | | between 1.1 and 1.3 mg/kg |

*numbers followed by a different letter are significantly different

the thymol residue levels in the honey had increased. As there is no MRL (Maximum Residue Level) for thymol, the taste threshold was used as a maximum. The average as well as the maximum thymol residue level of the honey from colonies treated with Thymovar was below the taste threshold, which is between 1.1 mg/kg and 1.3 mg/kg (Bogdanov *et al.* 1999).

The variation in thymol residue levels between the honeybee colonies treated with Thymovar was significant (minimum 0.27 mg/kg, maximum 0.60 mg/kg). The variation in thymol levels between the honeybee colonies used as controls was minimal (minimum 0.02 mg/kg, maximum 0.06 mg/kg).

Variation in formic acid and thymol residue

The variation in residue levels was probably influenced by colony size en the size of the brood nest. These two factors affect relative humidity, temperature and air circulation within the colony, which have an effect on the evaporation of formic acid and of thymol from the Thymovar plates.

Conclusion

Both the formic acid treatment and the Thymovar treatment increased the levels of these substances in the honey in case a honey super was placed immediately after treatment.

The average formic acid residue level is below the taste threshold. The maximum measured formic acid residue level however, exceeds this taste threshold.

When formic acid treatment is used in spring, it should be taken in to account that formic acid residue could affect the taste of the honey.

The average thymol residue level in honey and the maximum measured thymol residue level in honey are below the taste threshold. Therefore, a Thymovar treatment before honey flow in spring does not affect the taste of the honey.

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