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## Residues in honey and wax after treatment of bee colonies with formic acid

*Rester i honning og voks efter behandling af bifamilier med myresyre*

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### Summary

Bee colonies without the mite *Varroa jacobsoni* were treated with formic acid in the autumn in order to investigate the concentrations in residual feed, honey and wax. Samples for the investigation were taken eight to nine months after the treatment.

25–51 mg formic acid/kg were found in honey from the treated bee colonies, while in the untreated colonies approximately 20 mg formic acid/kg were found.

Traces of formic acid were discovered in the wax amounting to 10 mg/kg.

**Key words:** *Varroa jacobsoni*, *Apis mellifera*, honey bees, formic acid, residual concentrations, honey, wax.

### Resumé

Bifamilier uden miden *Varroa jacobsoni* blev behandlet med myresyre om efteråret for at få kendskab til restkoncentrationer i foderrester, honning og voks. Prøver til undersøgelsen er udtaget otte til ni måneder efter behandlingen.

Hos de behandlede bifamilier er der i honningen fundet 25–51 mg myresyre/kg. Hos ubehandlede bifamilier blev der i honningen fundet ca. 20 mg myresyre/kg.

I vokset er der fundet spor af myresyre på omkring 10 mg/kg.

**Nøgleord:** *Varroa jacobsoni*, *Apis mellifera*, honningbier, myresyre, restkoncentrationer, honning, voks.

## Introduction

Honey bees (*Apis mellifera*) can be attacked by the mite *Varroa jacobsoni*. This mite has spread to honey bees in many parts of the world. In Central Europe, as in other places, experience has shown that bee colonies die after a few years unless treated (4). In the course of a few years, the mite is expected to have spread to bee colonies in many parts of Denmark.

At present, treatment is being carried out abroad by means of a number of chemical substances. Many of the substances have to be used in the autumn, when there is no brood in the bee colonies (3). As there is no actual nectar flow at that time of the year, the residual concentrations in the honey of the substances involved are reduced.

Formic acid is one of the substances that is effective in treating for *Varroa* (5). It is probable that this substance will be used in Denmark.

As is the case when using other chemical substances, treatment with formic acid should take place after the termination of the nectar flow. However, unlike certain other substances, formic acid can be used while there is sealed brood in the bee colonies as it also destroys the *Varroa* mites present in these cells (1).

In order to investigate the concentrations in residual feed, wax and honey after treatment with formic acid, bee colonies without *Varroa* mites were treated with this substance. This paper describes the experiments conducted.

## Method

### Treatment using formic acid

In autumn 1983, bee colonies were treated with formic acid at two separate locations on Zealand (St. Dyrehave and Pagterold). Six bee colonies were treated in the apiary in St. Dyrehave while four bee colonies were treated at Pagterold. Each colony was treated with 180 ml 98 p.c. formic acid, as described by Ritter and Rutner (5). With this method, the formic acid is put into flasks with gauze wicks. The flasks are then placed in the bee hives above the bees, when the formic acid evaporates, the bees distribute the fumes by ventilating with their wings. This treatment was carried out in the second half of September and the first half of October.

As a control in the treatment with formic acid, six untreated bee colonies were wintered in St. Dyrehave and four at Pagterold in 1983.

During the winter, one of the treated bee colonies at Pagterold died. In the middle of May 1984, samples of residual winter feed and wax were taken from three of the colonies treated in St. Dyrehave and two treated at Pagterold. As a control, samples were taken from one untreated colony at each location.

Samples were taken again at the beginning of July 1984. This time of honey from the first extraction from three treated colonies in each apiary and from one control colony in St. Dyrehave.

### Method of analysis

The analyses were carried out in accordance to the enzymatic method as described by the firm Boehringer-Mannheim (no.1182.109.2-(2)). The limit of detection is estimated to be 5–10 mg formic acid/kg honey.

In the presence of FDH (formiatedehydrogenase), formic acid reacts with NAD (nicotinamide-adeninedinucleotide). Thereby NADH, which is the reduced form of NAD, is formed. The absorbance at the wave-length maximum ( $\lambda$  max) for NADH (334 nm) is measured. The reaction takes place directly in a cuvette where 0.5 ml honey diluted in the proportion 1:5 is added. The reaction time is 45 minutes.

## Results

In Table 1, the results following treatment with formic acid are shown. In the apiary in St. Dyrehave, the temperature was too low for total evaporation of the formic acid to take place and only 80–140 ml evaporated here. At Pagterold, all the formic acid evaporated in three of the colonies. In the fourth colony (no. 404), sugar water seeped into the flask containing the formic acid, resulting in only 20 ml evaporated.

In the residual feed, 41–209 mg formic acid/kg were detected. In the honey, 25–51 mg/kg were discovered. In the two colonies used as controls, a natural concentration of formic acid was found in the residual feed and in the honey in an amount about 20 mg/kg.

To evaluate these figures, Danish honey bought at random was examined, and here 38 mg formic acid/kg was found to be present.

In the wax component of the residual feed, traces of formic acid (about 10 mg/kg) were found.

**Table 1.** Concentrations of formic acid in residual feed in May and in honey in July 1984 after treatment with formic acid in September/October 1983

*Koncentrationer af myresyre i foderrest i maj og i honning i juli 1984 efter behandling med myresyre i september/oktober 1983.*

Apiary	Bee colony no.	ml formic acid evaporated	mg formic acid/kg residual feed	mg formic acid/kg honey
St. Dyrehave	141	140	41	n.a.
	15	120	127	n.a.
	101	100	n.a.	41
	3	90	n.a.	n.a.
	177	90	n.a.	51
	12	80	92	25
	Control	0	22	20
Pagterold	333	180	209	41
	315	180	n.a.	31
	25 *	180	n.a.	n.a.
	404	20	91	34
	Control	0	20	n.a.

n.a. = not analysed

\* = bee colony died during the winter following treatment.

The limit of detection is estimated to be 5–10 mg formic acid/kg.

One of the ten bee colonies of the untreated control group died during the winter, as did one of the colonies which were treated with formic acid.

## Discussion and conclusion

A certain amount of formic acid may be present naturally in honey. This was shown in an extensive investigation of German honey, where from 0 to 123 mg formic acid/kg were found occurring naturally in flower honey (6). The average formic acid concentration was 27 mg/kg. In honeydew honey, the concentration was as high as 600 mg/kg.

In another German investigation, levels of up to 85 mg formic acid/kg honey were found occurring naturally (2). In the same investigation, samples of winter feed and honey from bee colonies that had been treated with formic acid were examined. A maximum of 420 mg formic acid/kg was found in the honey, but in some of the samples nothing more than traces of formic acid were discovered.

In our investigation, approximately 20 mg formic acid/kg were found in the honey extracted from the bee colonies which had not been treated

with formic acid. A Danish honey bought at random contained just under 40 mg formic acid occurring naturally.

Approximately nine months after the treatment, 25–51 mg formic acid/kg honey was found to be present in the honey extracted from the treated bee colonies. Although in certain cases more than double the amount was discovered in the honey extracted from the treated colonies compared with the control colonies, it appears that the formic acid content in the honey of the treated colonies is approximately equivalent to formic acid levels occurring naturally in honey.

During the investigation no significant loss of bees was experienced. The loss of one bee colony in the group under treatment and of one from the control group in the course of the winter can be attributed to normal winter loss.

The results from this investigation were used by the National Agency of Environmental Protection when the authorization of the use of formic acid in the treatment of bee colonies infested by *Varroa* was under consideration. The National Agency of Environmental Protection has now given limited authorization for its use.

Translation into English by *Bodil Sampson*.

## References

1. *Adelt, B. & Kimmich, K.* 1986. Die Wirkung der Ameisensäure in die verdeckelte Brut. *Allg. dt. Imkerztg.* 20, 382–385.
2. *Koeniger, N., Held, T. & Vorwohl, G.* 1981. Mögliche Auswirkungen von Ameisensäurebehandlung auf den Honig. *Diagnose und Therapie der Varroatose*, 125–127.
3. *Ritter, W.* 1981. Varroa Disease of the honey bee *Apis mellifera*. *Bee World* 62, 141–153.
4. *Ritter, W.* 1986. Die Varroatose der Honigbiene, *Apis mellifera*, und ihre Bekämpfung mit Perizin. *Veterinär Medizinische Nachrichten* 1, 3–16.
5. *Ritter, W. & Ruttner, F.* 1980. Ameisensäure – Labor und Freilandversuche. *Allg. dt. Imkerztg.* 14, 151–155.
6. *Stoya, W., Wachendörfer, G., Kary, I., Siebentritt, P. & Kaiser, E.* 1986. Ameisensäure als Therapeutikum gegen Varroatose und ihre Auswirkungen auf den Honig. *Deutsche Lebensmittel-Rundschau* 82, 217–221.

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