ACIDIC FOOD AND NOSEMA DISEASE

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Abstract

It is not unusual that beekeepers add acetic acid to the winter food. It is effective for preventing formation of mould in feeders and may also have other effects. The chemical composition of the food may have an impact on the spore germination of the intracellular parasite Nosema apis*, but there are contradictory results on the impact from acidified food on nosema infections. We have studied the effect from acidic food on* Nosema *development in a field study and in laboratory experiments. Bee colonies (N=82) in the field were given winter food with different concentrations of acetic acid. Samples of adult bees from each colony were investigated for nosema disease in the fall at the time of feeding and in the following spring. Bees (N=225) in the laboratory were individually fed the same solutions as in the field study, but with addition of 10.000* N.apis *spores per bee. Control bees (N=75) received sugar solution or acidified sugar solution only. Samples were taken 4, 8 and 12 days post infection and the amount of spores in the midgut were counted in a haemcytometer. In a second experiment, also with addition of 10.000* N.apis *spores per bee but using only the highest concentration of acetic acid compared to non-acidified sugar solution, the rate of infected bees was investigated (n=210). No effect from altering the pH by addition of acetic acid could be found neither on the quantitative disease development of the parasite, nor on the infection rate of individual bees. The results from the field experiment support the laboratoty results;acidification of the food of honey bees has no influence on* Nosema *prevalence or development.*

Keywords: Nosema apis */ acetic acid / winter food*

Introduction

The microsporidian parasite *Nosema apis* infects the epithelial cells of the ventricles of the honey bee (*Apis mellifera)* (BAILEY, 1972; GRAAF, 1991). *N. apis* has spread world-wide (NIXON, 1982) but is not considered an important problem in tropical and sub-tropical climates (WILSON and NUNAMAKER, 1983). In temperate climates, infections by *N. apis* must be considered a serious disease. *N. apis* has a large negative effect on the production capacity of honey bee colonies in temperate climates (FARRAR, 1947; FRIES, 1984) and the survival of the colony during winter is affected by the disease (FARRAR, 1942; FRIES, 1988a). The problem with supersedure of infected queens adds to the economic damage caused by the parasite (FARRAR, 1947).

The addition of acetic acid into winter food may have positive effects in preventing different diseases. An experiment in Norway stated that acetic acid in the food reduced the occurrence of chalk brood (PEDERSEN, 1981), but the results have not been repeated. Laboratory experiments in Belgium suggest that acidified food decreases the development of *N. apis* in the midgut (MOTTOUL, 1996), but field studies performed in France demonstrated no impact from acidified food on nosema development (VAILLANT, 1989). The chemical composition of the food may have an impact on the spore germination of *N. apis.* When the spore enters the midgut of the bee, it germinates under the influence of the gut juices. Many chemical stimuli cause germination in vitro (LAERE, 1977) and it is possible that changing the chemical environment (i.e. lower the pH) in vivo could have an impact on spore germination. On the other hand, the pH-value of the honey is very low, 3.2 - 4.5, averaging about 3.9 (CRANE, 1975).

The aim of this experiment was to study the effect from acidic food on the development of *N.apis* under laboratory conditions and in the field.

Materials and Methods

Field studies

82 colonies in 8 different bee yards were randomly treated in three different ways in the fall 2002 at the time of feeding.

1. Sugar solution 2:3 w/v
2. Food consisting of sugar solution 2:3 w/v in addition of 2 ml conc. acetic acid/1000 ml
3. Food consisting of sugar solution 2:3 w/v in addition of 4 ml conc. acetic acid/1000 ml

In connection with the feeding, bee samples were taken to determine the occurrence of *N.apis,* and the pH-value in the food from a number of colonies in the different categories of treatment were measured.

Laboratory experiment I

Adult bees were individually fed (10 µl per bee, 30 bees per treatment) with the same sugar solutions as in the field study, but with the addition of 10000 spores of *N. apis* per 10 µl in following combinations (Table I) As shown, the spores were distributed either in acidified food or in sugar solution, followed by feeding either with sugar solution or acidified food.

Table I

Combination of treatment (group number), 30 bees per treatment, experiment I

Initial treatment	Additional treatment		
	Sugar solution	Acid 1	Acid 2
Sugar solution + spores	1	2	3
Acid 1 + spores	4	5	6
Acid 2 + spores	7	8	9
Sugar solution only	10	11	12

Sugar solution (sugar: water 3:2, pH 7.91)
Acid 1 (sugar solution + 0.2% acetic acid, pH 3.55)
Acid 2 (sugar solution + 0.4% acetic acid, pH 3.19)
The bees were incubated at + 30°C in 50% RH, with constant access to food. Five bees per treatment were examined 4, 8 and 12 days after treatment, and the number of spores in the midgut were counted in a hemocytometer. Twelve days after treatment, the rest of the bees were killed and examined for nosema.

Laboratory experiment II

To refine eventual impact from acidified food on *N. apis*, yet another separate experiment were conducted. In this experiment bees were fed spores initially in sugar solution, followed by additional feeding of sugar solution, or spores in acidified food followed by additional feeding with acidified food only. Two groups of bees were only fed sugar solution and acidified food respectively. These bees were used as control groups. (Table II).

Table II

Combination of treatments and number of bees per treatment, experiment II

Treatment	Number of cages	Bees/cage	Total number of bees
Sugar solution	1	15	15
Acid 2	1	15	15
Sugar solution + spores	6	15	90
Acid 2 + spores	6	15	90

The bees were individually fed (10 µl/bee) with sugar solution or acidified food with addition of 10000 spores of *N.apis* per 10 µl. The spore solutions had been refrigerated for one week. All of the bees were killed and examined for nosema 14 days after treatment.

Results

Laboratory experiment I

No impact on the quantitative development of *N.apis* from acidified food could be traced in from any of the treatments compared to controls in this experiment (figure 1).

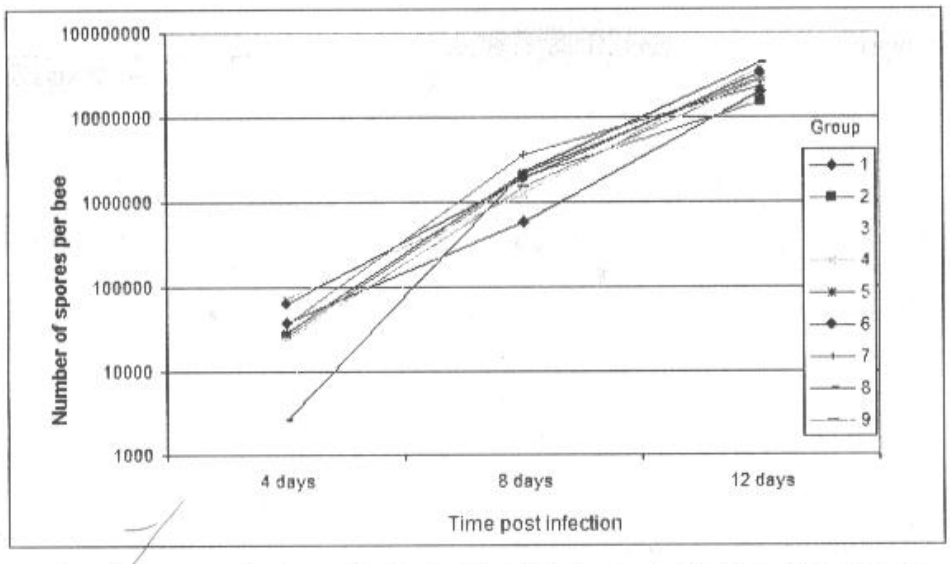

Figure 1 – Average number of spores found in the midgut of five bees examined for N. apis*, 4, 8 and 12 days post infection*

The amount of infected bees from the different treated groups is shown in figure 2. Bees examined 4 days post infection are represented in the graph but not part of the comparison calculations (KRUSKALL-WALLIS) because not all infected bees have traceable amounts of spores already 4 days post infection (FRIES, 1988b).

In figure 2 the number of infected bees in the respective groups are shown. There is no significant difference in the proportion of infected bees between the different treatments (Chi-square, p›0,05).

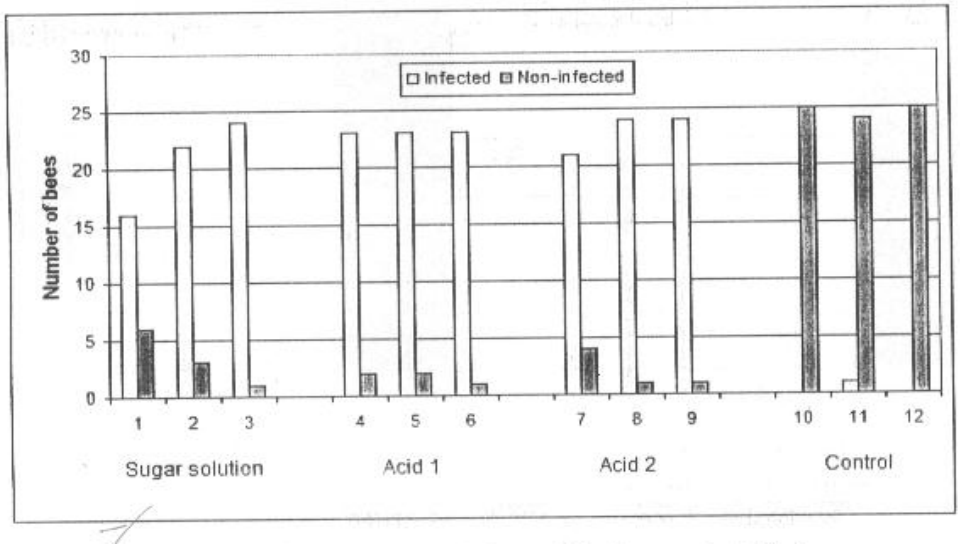

Figure 2 – The number of infected bees in experiment I. Group numbers corresponds to table I

Laboratory experiment II

In this experiment, the highest concentration of acetic acid was compared to non-acidified sugar solution. Data demonstrate no reduction in the proportion of infected bees when spores are fed in acidified food and bees continued to be fed acidified food post infection (Chi-square, p›0,05).

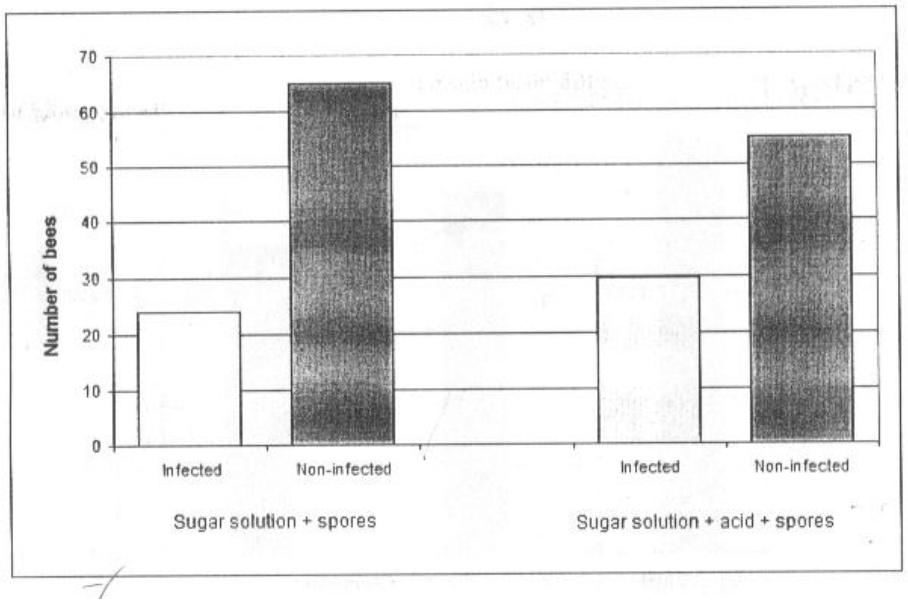

Figure 3 – The number of infected bees in experiment II. The treatment of the two groups is specified in table II

Field experiment

The proportion of infected hives in the fall of 2002 is shown in figure 4. Neither in the fall nor in the spring is the difference between treatments significantly different from the controls (Chi-square, p›0,05). Figure 5 shows the average reduction of spores per bee from the fall of 2002 to the following spring, calculated as percentage. There is no significant difference between the (unexpected) reductions of spore levels between any of the groups.

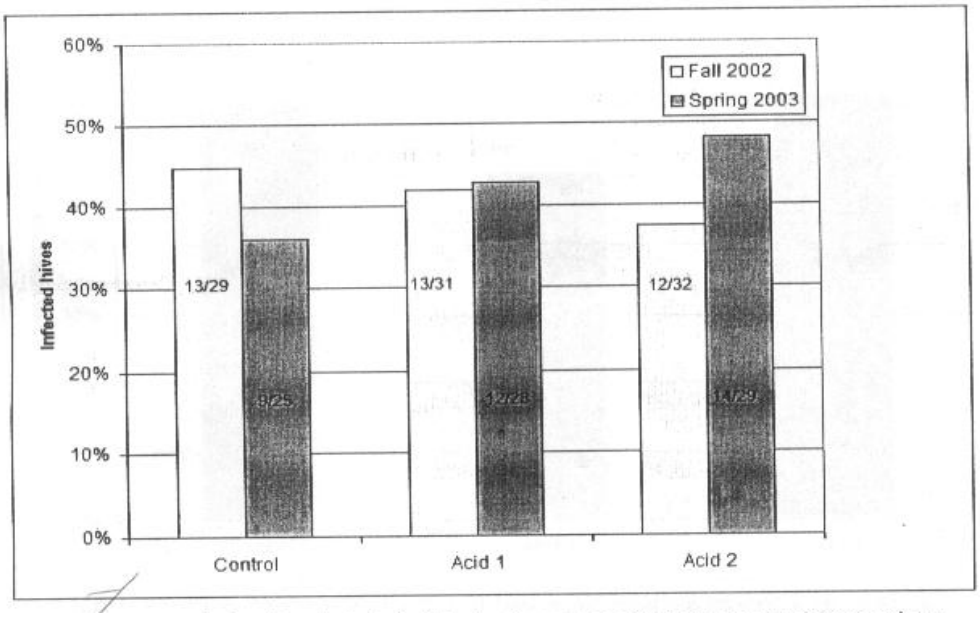

Figure 4 – Proportion of infected bee colonies in the fall and spring respectively in the control group fed sugar solution and the two groups fed different concentrations of acidified food. Numbers in bars correspond to number of infected hives over total number of hives

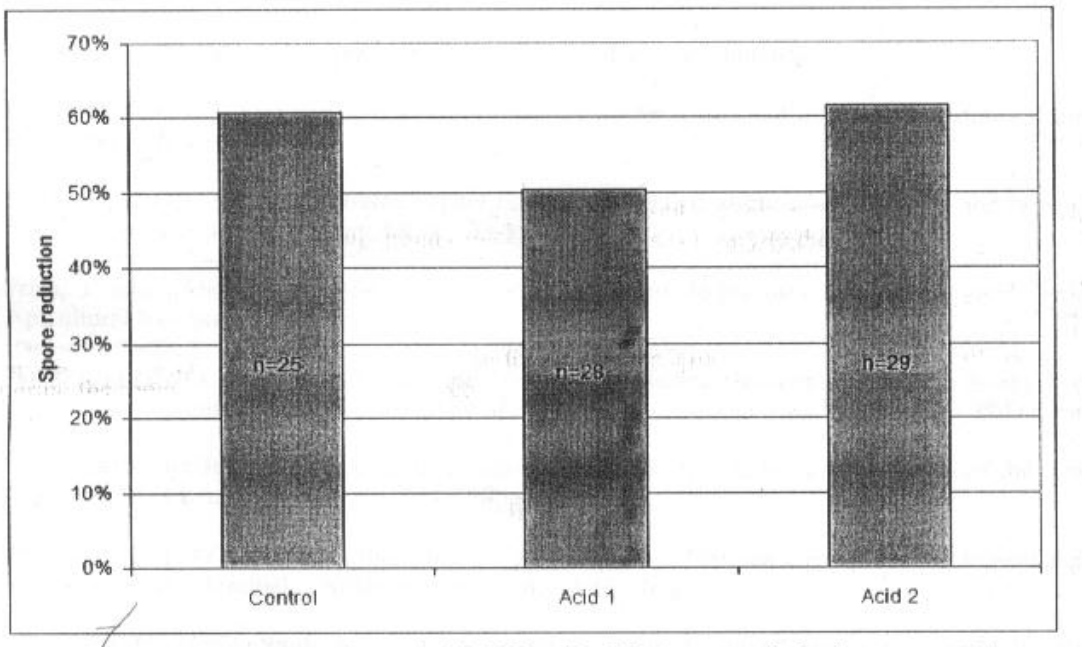

Figure 5 – Average reduction of spores between the fall of 2002 and the following spring, calculated as percentage (%)

Discussion

The laboratory results demonstrate that the infectivity or quantitative development of *N. apis* in honey bees is not influenced by the acidity of the food when spores are consumed. This conclusion is valid irrespective of weather the spores are consumed in acid solution and then bees are fed normal sugar solution, if spores are fed in sugar solution and bees then given acid solution, or if spores are fed in acid solution and bees then fed acid solution.

The results from the field experiment support the conclusions made from the laboratory experiments. From figure 4 can be seen that the trend (non-significant) is contrary to the hypothesis that acidification of food will lower the incidence of nosema disease. Thus, the field results support the conclusions of the laboratory experiments.

It is interesting to note that the proportion of infected hives actually has decreased from the fall to the spring in this experiment (Figure 4), and that there has been a reduction in the number of spores per bee during the same time (Figure 5). This is contrary to what can be expected (BAILEY & BALL, 1991) but the trend is similar in all groups and remains unexplained.

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