CONTROL OF NOSEMOSIS WITH VITAFEED GOLD

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ABSTRACT

VitaFeed Gold, a new product against nosema disease, was tested in naturally infested colonies during spring and autumn of 2006. During each period 10 infested colonies were used. In addition, Fumadil B was applied in another ten colonies and results examined comparatively. Equal numbers of untreated colonies served as control.

One month after medication, Nosema spores were not found in seven colonies treated with VitaFeed Gold in spring, and in eight treated in autumn. Most of the other colonies had a noticeable reduction of infection. Fumadil B gave comparative results. Colonies treated with VitaFeed Gold and Fumadil B had an increased number of brood and bee population while control colonies had a reduction in both parameters.

INTRODUCTION

Nosema disease, caused by the protozoan *Nosema apis* Zander, is the commonest disease of adult bees. *Nosema apis* develops within the epithelial cells of the midgut, directly affecting the physiological function of the digestive tract. Under favourable conditions for its spread, an infected colony may become seriously weakened during the build-up period in spring. The disease generally subsides with the coming of warm weather.

Nosema is a chronic, insidious disease of honey bee colonies throughout most of the world. The disease is endemic in many apiaries and may be present in a colony without causing noticeable damage.

The endemic character of Nosema is more intense in Greece for several reasons. The majority of Greek beekeepers move their colonies to pine-forests to collect the abundant secretion of honeydew between September and November each year. Since the availability of pollen in pine-forests is low, wintering bees usually have protein deficiency - which affects the impact of Nosema disease (Bailey 1953). In addition, the honeydew honey, which is the predominant store for over-wintering colonies, has more residual matter (dextrin) than floral honey, and this causes great problems especially when the weather does not permit cleansing flights for defecation in the cold months of January and February.

Colonies in more temperate areas, like Greece, are normally more active during winter, rearing brood, increasing the temperature in their hives and consuming more honey. As a result, more faeces is accumulated in their rectum. This is an additional factor that contributes to the development of the disease. Furthermore, because of the mild weather, beekeepers open and manipulate colonies during winter more frequently than in other countries. Colonies of honeybees that are opened and manipulated at regular intervals during winter show more infection with *N. apis* than others that were not opened.

For the control of Nosema, the antibiotic fumagillin of the commercial product Fumadil B has been used extensively (Moffet et al.1969). The European Medicines Evaluation Agency (EMEA) adopted Council Regulations (EEC) 2377/90 and 434/97 that require Maximum Residues Limit (MRLs) for all pharmacologically effective substances from veterinary drugs in foodstuffs. Based on these regulations, the use of antibiotics that lack MRLs has been illegal in beekeeping since January 2000. No MRLs have been set for fumagillin, but the committee for Veterinary Medicinal Products (CVMP) added Fumadil B to the list of essential substances for the treatment of Nosema diseases in bees where no alternative product exists (EMEA/CVMP/411/00 FINAL).

The provisional permission of Fumadil B expired in December 2005. Since then, bees remain defenseless against Nosema and beekeepers are desperately seeking solutions. New products should be developed against Nosemosis - containing no antibiotics and no chemicals, to have the potential to get permission from EMEA. Chioveanu et al. (2004) tested with success a natural product against Nosema under the commercial name Protofil. Several other products have appeared also through Internet websites (NONOS, NOSESTAT) (www.beekeeping.com/anivet/ondex.htm).

In the present paper we tested VitaFeed Gold as a promising solution against Nosema in naturally infested colonies during spring and autumn. VitaFeed Gold is a water extract of *Beta vulgaris* cv *altissima* rich in sodium salicylate and molasses. This product was developed by Vita (Europe) Ltd as a stimulant of honeybee population.

MATERIAL AND METHODS

Experimental colonies: The effectiveness of the treatments was assessed in 30 natural infested colonies during spring (April-May) and in another 30 colonies during autumn (October-November) of 2006. The experimental colonies were selected, according to their infestation level of nosemosis, from two different apiaries of *Apis mellifera macedonica* located in areas close to Thessaloniki in northern Greece.

Grouping: Based on infestation level and strength of the colonies, 3 batches of 10 colonies each were formed. Batches A and B were treated with VitaFeed Gold and Fumadil B respectively and batch C served as control.

Administration of therapeutic agents: VitaFeed Gold was diluted in sugar syrup (50% water, 50% sugar) to make a 10% solution. Each colony received 10 ml solution per frame population by dribbling evenly between the bee spaces every other day (on days 1, 3, 5, 7, and 9). The full course of treatment was 500 ml diluted solution for a 10-frame population colony, over 9 days.

Twelve and half grams of Fumadil B were diluted in 5 L of syrup. Each colony was supplied 50 ml therapeutic syrup per frame population. Three therapeutic treatments with weekly intervals were made.

Control colonies were fed with 10 ml syrup per frame population every two days.

Sampling and counting of nosema spores: Approximately 100 bees were collected from the entrance of naturally infested colonies using a vacuum-powered collection device and were analysed in laboratory for the presence of nosemosis.

The counting of nosema spores was achieved according to Cantwell's (1970) protocol as follows:

a) The abdomen of ten worker honey bees was placed in a mortar with 5 ml of water and ground with the rounded end of a pestle.

b) The suspension was filtered through two layers of muslin (thin loosely woven cotton fabric) in a funnel. A second 5 ml of water was used to rinse the pestle, swirl around the inside of the mortar and pour through the sub sample in the funnel. The suspension was finally poured into a graduated centrifuge tube.

c) The weight of tubes was equalized with water and the suspensions were centrifuged for 6 minutes at 800 g (3200 rpm).

d) The supernatants were decanted and the tubes were refilled to the 10 ml level.

e) Using disposable pipettes, the pellets were re-suspended by repeated uptake and forcible ejection through the pipette tips.

f) When the solution appeared to be homogenous, a sample was taken to fill the calibrated volume under the cover-slip of a haemocytometer. After three minutes the spores have settled to the bottom of the chamber.

g) A magnification of X400 and bright-field of an Olympus microscope BX40 was used to count the number of spores in each square (fig. 1).

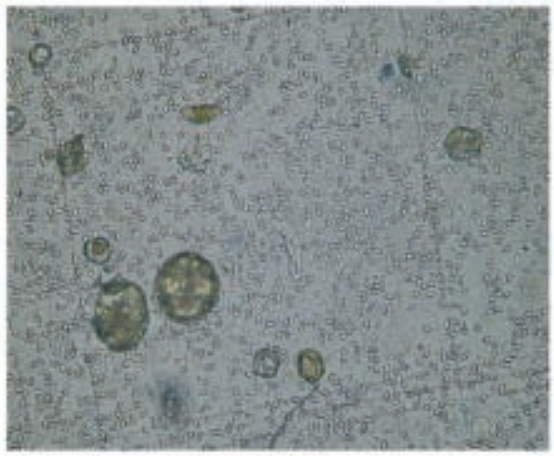
h) One nosema spore, observed in the area that covers the entire etched grid, is equal to an average of 4 million spores per bee. We counted at least 16 squares per sample.

i) If no spores were seen the results was designated “not found.”

The efficacy of applied methods was calculated by the decrease of the level of infection with Nosemosis in experimental colonies one month later.

Impact of treatment on bee. The strength of the colonies, the brood area and the presence of a laying queen was noted before and after the experiment. Bee mortality was monitored daily by putting a dead bee trap in front of each hive.

Figure 1. Counting the number of nosema spores in the haemocytometer



RESULTS AND DISCUSSION

During spring medication, seven infected colonies treated with VitaFeed Gold were cured and no spores were found in the examined bees. The other three colonies had low infestation. During autumn treatment, eight colonies were cured with VitaFeed Gold and two colonies had 32% and 38% reduction of nosema spores respectively (table 1).

Colonies treated with Fumadil B presented similar results. Samples of bees collected from six colonies, treated with the antibiotic during spring, had no detectable spores while the reduction of spores in the rest ranged from 30.0% to 97.3% (table 1). During autumn treatment, Fumidil B gave better results. Eight colonies were found without nosema spores and two had noticeable decrease of infection.

The course of infection in the control group was different during the two seasons. During spring, three colonies were self cured and most of the others had a perceptible reduction of infection. These results were expected since April and May are the months with high temperatures and plenty of pollen sources and available nectar. During this period, bees received lot of nourishment and the emerged young bees were more resistant to nosema. In addition, the ability to taking cleansing flights outside of the hive helped to reduce the infection. During autumn, in one colony nosema spores were not found (colony 3) while in another one the spores were dramatically decreased (colony 5). In the remaining colonies the spore numbers increased.

Table1. Efficacy of therapeutic agents against nosema disease

	Spring treatment			Autumn treatment		
	Spores of Nosema x 10^6			Spores of Nosema x 10^6		
	15/4/06	16/5/06	% changes	20/10/06	20/11/06	% changes
VitaFeed Gold treatment						
1	54,1	N.F	-100	684,6	418,6	-38,0
2	325,0	2,3	-99,0	98,0	N.F.	-100
3	65,5	1.2	-98,1	50,6	N.F	-100
4	19,2	N.F	-100	187,3	N.F	-100
5	12,0	N.F	-100	175,3	N.F	-100
6	44,3	N.F	-100	69,0	N.F	-100
7	30,6	2,5	-91,8	100,6	1,3	-99,0
8	15,3	N.F	-100	106,6	N.F	-100
9	27,3	N.F	-100	190,0	132,6	-32,2
10	8,3	N.F	-100	15,6	N.F	-100
Fumadil B treatment						
1	106,2	10.2	-90,4	14,6	6,6	-54,7
2	15.2	N.F	-100	20,0	N.F.	-100
3	10.8	N.F	-100	6,7	N.F	-100
4	20,3	14,2	-30,0	229,0	N.F	-100
5	23,3	N.F	-100	133,3	N.F	-100
6	115,0	3,1	-97,3	166,6	N.F	-100
7	19,3	N.F	-100	168,0	N.F.	-100
8	12,0	N.F	-100	6,0	N.F	-100
9	32.7	N.F	-100	69,3	N.F	-100
10	33,4	12,5	-62,5	159,9	13,4	-91,6
Control						
1	20,3	18,3	-9,9	150,6	201,3	+33,6
2	23,5	10,3	-56,2	23,4	172,0	+635,0
3	225,0	35,7	-84.1	35,3	N.F	-100
4	56.9	34,3	-39,8	64,0	305,0	+376,0
5	13,9	N.F	-100	181,3	5,3	-97,2
6	34.8	2,3	-93,4	4,6	35,7	+676,0
7	17,3	N.F	-100	3,9	45,8	+1074,3
8	12.8	N.F	-100	128,9	250,6	+94,1
9	23.9	3,8	-84,2	230,5	380,8	+65,2
10	12,0	10,6	11,5	23,6	50,2	+112,7

-: decreased number of spores
+: increased number of spores

Figure 2 shows the average number of dead bees in the traps in front of the experimental colonies during spring. In all cases the number of dead bees in front of the hives decreased with time. By the end of the treatment in all the cases the number of dead bees reached normal levels (0-10 dead bees/day/colony)

Figure 2. Daily number of dead bees collected from the entrance of the experimental colonies during spring.

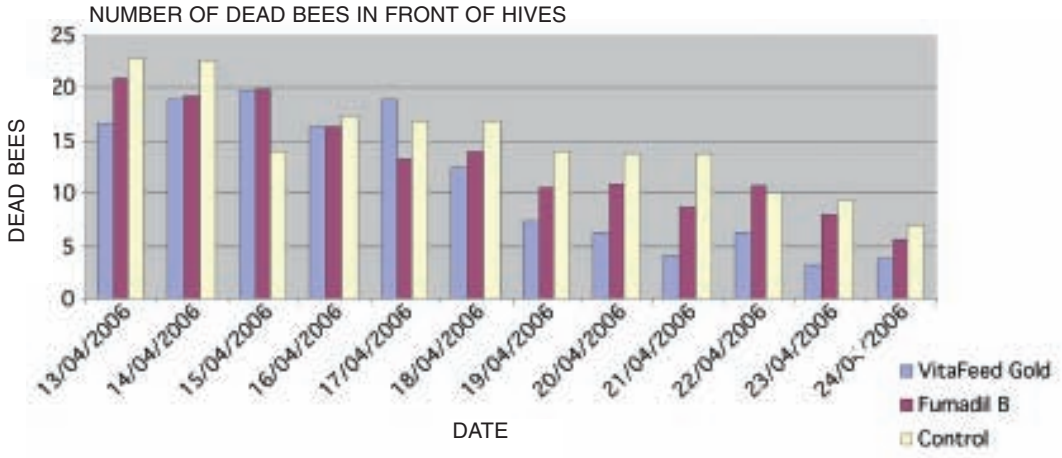


Table 2 indicates the average number of frames with brood and population in experimental colonies before and after treatment. Colonies treated with VitaFeed Gold or Fumadil B had an increased number of combs with brood in spring, while control had a reduction. The population of colonies treated with VitaFeed Gold and Fumidil B expanded faster than the control colonies. During autumn both brood and population decreased in all the experimental colonies. The decrease was larger in the control colonies than the treated ones (table 2).

Table 2. Changes of brood and bee population in experimental colonies

	Total number of frames with brood in 10 colonies			Total number of frames with bee population in 10 colonies		
	before treatment	after treatment	changes %	before treatment	After treatment	changes %
Spring treatment						
VitaFeed Gold	42	52	+23,8	69	83	+16,8
Fumadil B	42	48	+14,2	67	78	+16,4
Control	40	39	-2,5	68	70	+2,9
Autumn treatment						
VitaFeed Gold	30	14	-53,3	68	63	-7,3
Fumadil B	27	17	-37,0	61	58	-4,9
Control	28	9	-67,8	66	54	-18,2

These results indicate that VitaFeed Gold is well tolerated by bees, and it has a stimulating effect on the development of the bee colonies. The decreases of *Nosema apis* spores after 9 days administration indicates a promising therapeutic effect of the medicine. The mode of action is not well known. The water extract of *Beta vulgaris* cv *altissima* is rich in sodium salicylate which has many properties, bactericide, fungicide, and antioxidant. It also kills the amoeba spores. The stimulating effect of VitaFeed Gold is probably due to the extract of *Beta vulgaris* cv *altissima* which is rich in amino acids, macro- and micro-elements, vitamins, and sugars.

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