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Original article

The stability and effectiveness of fumagillin in controlling *Nosema ceranae* (Microsporidia) infection in honey bees (*Apis mellifera*) under laboratory and field conditions

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Abstract – Honey bees play an important role in food production (honey, pollen etc.), and their pollinating activity is not only essential to maintain world agriculture production but also to ensure biodiversity in different ecosystems. *Nosema ceranae* is a highly prevalent worldwide pathogen for honey bees that has been related to colony losses. A commercial formulation that contains fumagillin dicyclohexylamine, Fumidil B®, can control *N. ceranae* infection. However, the effectiveness of Fumidil B® is affected by several factors, such as storage, treatment preparation, the quantity consumed by bees etc. Indeed, UV exposure (e.g. sunlight) drastically reduces the initial concentration of fumagillin within a few hours, while temperature affects its degradation. Although laboratory tests suggest that a semisolid mixture of honey and powdered sugar is the best option to apply fumagillin, its application in syrup (250 mL per dosage) is more effective for the treatment of infected colonies. The total amount of syrup containing fumagillin ingested by honey bees is a key factor in its efficacy, and it has been found that medicated patties were not fully consumed in field trials. In honey bee colonies, the dose of 120 mg/honey bee colony at the recommended posology is effective against depopulation and colony death due to *N. ceranae* after 1 year, without residues being detected in honey, although reinfection could be detected 4 months after treatment ended.

Nosema ceranae **/ Fumagillin / Stability / Syrup / Honey–sugar patty / Dosage / Treatment / Colony depopulation**

1. INTRODUCTION

The treatment of infectious diseases in food-producing animals is an essential aspect of veterinary medicine. Likewise, it is necessary to treat honey bee diseases to maintain their role as food producers (honey, pollen etc.) and as pollinators of crops and wild vegetation. Indeed, it was estimated that the worldwide economic value of the pollination services provided by insects in 2005 was about 153 billion € (Gallai et al. 2009), disregarding their essential role in maintaining the biodiversity of ecosystems.

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Microsporidia are parasites considered to infect all animal phyla. *Nosema ceranae* (Fries et al. 1996) is a microsporidium found worldwide and it is considered to be one of the main honey bee pathogens (Higes et al. 2006, 2008; Chen et al. 2009; Cornman et al. 2009). Since it was first detected outside Asia (Higes et al. 2006), this microsporidium has been identified in many continents (Chauzaut et al. 2007; Cox-Foxter et al. 2007, Klee et al. 2007; Huang et al. 2007; Martín-Hernández et al. 2007; Williams et al. 2008; OIE 2008; Higes et al. 2009a).

N. ceranae infects midgut epithelial cells of adult worker honey bees and queens (Higes et al. 2007, 2009b), causing irreversible damage. It is pathogenic when inoculated experimentally into *Apis mellifera* (Higes et al. 2007; Antúnez et al. 2009; Chen et al. 2009), as well as in naturally infected honey bees (Higes et al. 2008) or in commercial honey bee colonies (Higes et al. 2009c; Korpela 2009; R. Borneck, unpublished data; M.J. Valerio, unpublished data). It has also been suggested that *N. ceranae* induces significantly higher mortality than *N. apis* (Paxton et al. 2007; Martín-Hernández et al. 2009; Higes et al. 2010a), the latter being considered to be the sole etiological agent responsible for nosemosis until *N. ceranae* was related to a different clinical pattern of nosemosis (Higes et al. 2008, 2010b). More recently, it was demonstrated that natural *N. ceranae* infection causes honey bee colony collapse and death, and moreover, that the use of fumagillin dissolved in syrup could be used to treat the infected colonies in field conditions (Higes et al. 2008, 2009c).

Fumagillin is obtained from *Aspergillus fumigatus* (Bailey 1953; Webster 1994; Didier 1997) and it is one of the few drugs know to be active against microsporidia (McCowen et al. 1951). Indeed, it has been used to treat *N. apis* in honey bees since the early 1950s (Katznelson and Jamieson 1952; Bailey 1953), and this approach has been adapted to fight against microsporidiosis in other insect species. However, fumagillin is not always successful in combating microsporidia and it is ineffective against *Nosema bombi* in bumble bees (Whittington and Winston 2003).

Since 2002, the European Medicines Agency has considered fumagillin as an "orphan drug" for the treatment of human diarrhoea associated with intestinal microsporidial infection (EMEA 2000, 2003). In addition, it is the only chemical registered in USA and Canada to treat *Nosema* infection in honey bees. In recent years, in several European countries (UK, Spain, Belgium, Greece, Hungary, Romania etc.) exceptional temporary authorization has been given to use fumagillin under veterinary supervision to treat nosemosis in positively diagnosed apiaries. This situation has been brought about due to the impact of bee losses and the high prevalence of colonies infected by *N. ceranae*.

While applying recommended posology in Spain, certain differences have been reported in the effectiveness of fumagillin in *N. ceranae*-infected honey bee colonies. For this reason, we have evaluated some factors that may influence the efficacy of this antibiotic in field conditions, as well as the stability of fumagillin and the presence of residues in honey from treated colonies.

2. MATERIALS AND METHODS

2.1 Fumagillin stability

The fumagillin excipients selected for this study were syrup and patty, since they are those most frequently used by beekeepers to apply medicines or to feed honey bee colonies. Sucrose syrup was prepared by mixing sugar (commercial grade, Ebro Agrícola, Valladolid, Spain) with ultrapure water in equal parts. The honey–sugar patty was prepared by vigorously mixing multifloral honey from La Alcarria Appellation of Origin (Guadalajara, Spain) with powdered sucrose, also in equal proportions. This honey has been analysed previously to confirm that it did not contain any fumagillin residues (Nozal et al. 2008).

Syrup and honey–sugar patty were prepared in sufficient quantities to give 300 aliquots, each of which was subjected to different conditions to try to simulate their use in honey bee colonies under field conditions. All the assays were carried out in triplicate.

Fumidil B® (commercial formulation, 20 mg of fumagillin/g, Ceva Santè Animale) was dissolved in ultrapure water (Milli-RO plus system and Milli-Q system: Millipore, Bedford, MA, USA). To study its stability, an Agilent Technologies (Palo Alto, CA, USA) 1100 series LC-DAD system with an automatic injector and column oven was used, all controlled by Chemstation software. High-performance liquid chromatography (HPLC) analysis was carried out on a 4.6×150 mm Gemini® chromatography column packed with C_{18}, 110 A, 5 μm particles, from Phenomenex (Torrance, CA, USA). The mobile phase in the isocratic mode was a mixture (2:3 *v*/*v*) of acetonitrile (HPLC grade, Lab-scan Ltd., Dublin, Ireland) and ammonium formate (20 mM Merck, Darmstadt, Germany). The flow rate and the injection volume were 1 mL/min and 20 μL, respectively. Since the high viscosity of the mixtures prevented the direct injection of the samples onto the HPLC system, 20 μL of a 5% dilution of both matrices in deionised water (mainly for honey–sugar patty samples) was injected for the analysis.

The UV-spectrum of Fumidil B® was very similar (between 335 and 355 nm) and in accordance with previous reports (Brackett et al. 1988; Assil and Sporns 1991; Kochansky and Nasr 2004), 350 nm was the wavelength selected for a sensitive detection.

2.1.1 Effect of temperature and UV radiation

Aliquots containing fumagillin or Fumidil B® were divided into groups of 40 units (20 stored in amber and 20 in clear vials) and they were maintained under different conditions: (1) refrigerated at 4°C; (2) at room temperature (22°C; (3) in a drying oven at 30°C; (4) in a drying oven at 40°C; and (5) under exposure to UV radiation (6 W short/longwave ultraviolet lamp, Cole-Palmer Int., Vernon-Hills, IL, USA). Aliquots from each group were analysed daily from days 0 to 10, and weekly up to day 100, and the results were compared with those obtained when the solutions were prepared.

2.1.2 Effect of fumagillin concentration

To determine the influence of dilution of the commercial product on fumagillin stability in the syrup and patty, three doses of Fumidil B® (1, 1.5 and 2.5 g) were added to 250 mL of each matrix and analysed in triplicate.

2.2 Field trials

2.2.1 Honey bee colonies and pathogens

The study was carried out between June 2006 and December 2007 (over 19 months) in 70 strong colonies of *A. mellifera iberiensis* located at an experimental apiary (Marchamalo, Central area of Spain, 40°40′57.47″ N, 03°12′3.08″ W). The colonies used in this study were acquired from a commercial beekeeper and the queens were all of the same age (1 year old). The colonies were analysed to confirm the absence of the principal bee pathogens (both adult bees and brood) when they were introduced into the apiary in June 2006 (OIE methods, 2004, modified). The honey bee colonies were located close to a *N. ceranae*-infected apiary. No genetically modified agricultural crops were cultivated in the surrounding areas, nor were neonicotinoids in use. The pollen sources in this area were present in sufficient quatities for the bees throughout the year. *Varroa* infestation was controlled twice annually during the study using amitraz (Apivar®) or flumetrin (Bayvarol®) to avoid the negative effect of this parasite on the health of the honey bee colonies. The absence of any brood pathology was confirmed at each visit to the apiary, and the presence of the queen was also confirmed to determine if replacement had occurred or not.

2.2.2 Determination of Nosema species

The determination of the *Nosema* species was carried out by means of duplex PCR using the 218MITOC FOR/218MITOC REV and 321APIS FOR/321APIS REV primers (Martín-Hernández et al. 2007). DNA was extracted as described in Higes et al. (2008).

2.2.3 Fumagillin treatment

In September 2006, prior to the onset of treatment with fumagillin, *Nosema* infection was confirmed by phase contrast microscopy and PCR (Martín-Hernández et al. 2007; Higes et al. 2008) in 30 forager honey bees from each of the experimental colonies

(n=70). The 70 colonies were randomly distributed into seven groups (Table I) according to the posology of fumagillin (as Fumidil B®, CEVA). The sample design was incidental (incidental sampling) but it represented well the variability of the data. Although the groups were of different sizes, representativeness was assured. The experimental groups were: three control groups "C" not administered Fumidil B; two groups starting with number 1 administered 6 g of Fumidil B per colony and two groups starting with two administered 8 g/per colony. Two excipients were tested with each concentration denominated as "S" for the syrup groups and "P" for the patty ones (mixtures prepared as described in the stability studies). One control group was included for each excipient plus a third one that received only syrup supplemented with pollen substitute (Promotor L, Lab. Calier).

The consumption of the preparations applied to each colony was assessed weekly. Each treatment started in October when all the colonies were *N. ceranae* positive, and they were maintained until November (groups 1S and 1P) or December (groups 2S, 2P, C1, C2 and C3), depending on the number of applications required (four or eight, see Table I). The effectiveness of the treatments was determined in December 06 (18 days post-treatment—p.t.), April 07 (6 months p.t.) and December 07 (13 months p.t.) by assessing the presence or absence of *N. ceranae* in forager honey bees. The health status of the colonies (evident through the absence of any clinical sign indicative of a pathology other than *Nosema* infection) was also recorded at each visit to the apiaries.

2.3 Analysis of fumagillin residues in honey after treatment

To determine the possible accumulation of fumagillin residues in the honey after treatment, samples from brood chamber combs were collected from four randomly selected hives in each group treated with Fumidil B® in January 2007 and from just two hives in the control groups. At the end of May 2007, samples were taken from the combs in the upper box that corresponded to the honey produced in spring 2007 by these colonies. Honey samples were collected individually in sterile dark containers after treatment and stored at −20°C until they were analysed as indicated previously with a limit of detection in the chemistry analysis of 1 μg/kg (Nozal et al. 2008).

2.4 Statistical analysis

The differences in the effectiveness of fumagillin treatment between each group was established by binary dependent variables such as infection on December 2006 and death on April or December 2007. The severity of the clinical repercussions was compared by considering the colony death and the date of death, as well as infection and the date of detection. Bivariate sub-tables were defined by a row variable (treatment) and column variables (infection and death). A Chi-square test with exact probabilities was performed to test the null hypothesis of the independence of the response to treatment. A one-way analysis of variance, Kruskal–Wallis and the Mann–Whitney U test

Table I. Fumagillin treatments assayed in the field trial.

Group	Number of colonies	Fumidil B per application (g)	Excipient per application	Number of applications	Total Fumidil B dose per colony (g)
1S	9	1.5	250 mL syrup	4	6
2S	9	1	250 mL syrup	8	8
1P	12	1.5	250 g patty	4	6
2P	18	1	250 g patty	8	8
C1	5	–	250 mL syrup	8	0
C2	5	–	250 g patty	8	0
C3	12	–	250 mL supplemented syrup	8	0

Syrup (50% sugar/water solution). Patties (50% sugar/50% fumagillin-free honey). Supplemented syrup (syrup with 2% of pollen substitute (Promotor L). Fumidil B (20 mg/g fumagillin). Preparations administered each week

were used to study the degree of infection in an ordinal scale. The dependent variables were the progress of colony death and the degree of infection and the fixed factor was the type of treatment.

A categorical principal components (CATPCA) and ordinal regression were used to asses whether the amount of ingested food influenced the interaction with the treatment in order to explain the time of colony death given that the response variable is an ordinal variable.

3. RESULTS

3.1 Fumagillin stability

The most significant observations obtained from the evaluation of the stability of the different aliquots under the conditions tested are summarized below.

3.1.1 Fumidil B® added to syrup

When samples stored at 4°C were studied, the changes in the stability of fumagillin were very similar for each of the three doses over time. Accordingly, there was a progressive decline that reflected a 30% loss after 70 days in clear glass vials (Figure 1), which was slightly reduced (12%) when the samples were stored in amber vials. In the samples stored at 22°C, there was a 60% decrease in fumagillin after 70 days, and storage at 30°C produced a similar decrease close to 65% after 70 days. However, when samples were stored at 40°C, the fumagillin content rapidly diminished and after 20 days the compound was practically undetectable (Figure 1).

Finally, when the effect of UV radiation was studied, we found that although in degradation occurred rapidly in amber vials and the rate of disappearance was initially high (Figure 1), fumagillin degraded more rapidly in clear glass vials and after 24 h a reduction of about 80% was evident, with no fumagillin detected after 10 days. The evolution of the degradation products was also obvious when UV radiation acted on the sample stored in amber vials, with a maximum close to the 10th day (see Figures 1 and 2 for the chromatograms showing the effect of temperature and UV radiation).

3.1.2 Fumidil B® added to the honey–sugar patty

In samples stored at 4°C, the degradation rate of fumagillin in patties was slower in clear glass vials than under similar conditions when in syrup, and a 10–20% loss was evident after 4 weeks

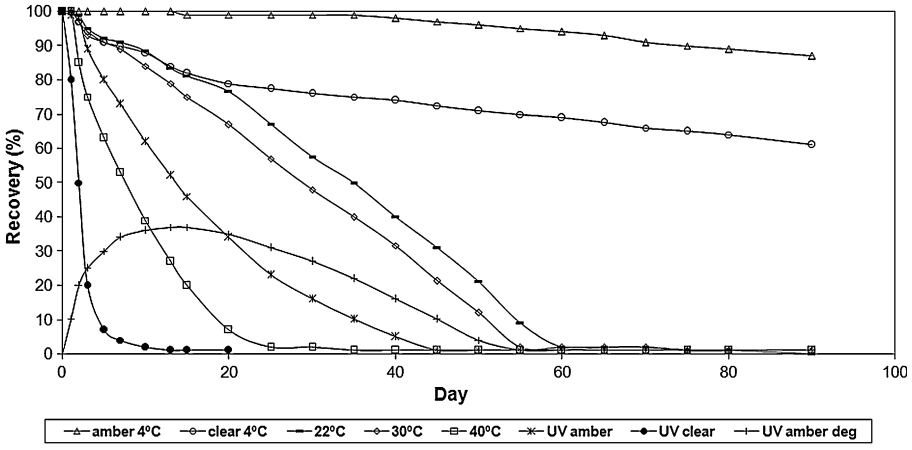

Figure 1. The influence of temperature, UV radiation and container type on the recovery of fumagillin from syrup spiked with 1.5 g of Fumidil B over time. The figure shows the data from one aliquot, representing the general tendency of all the samples.

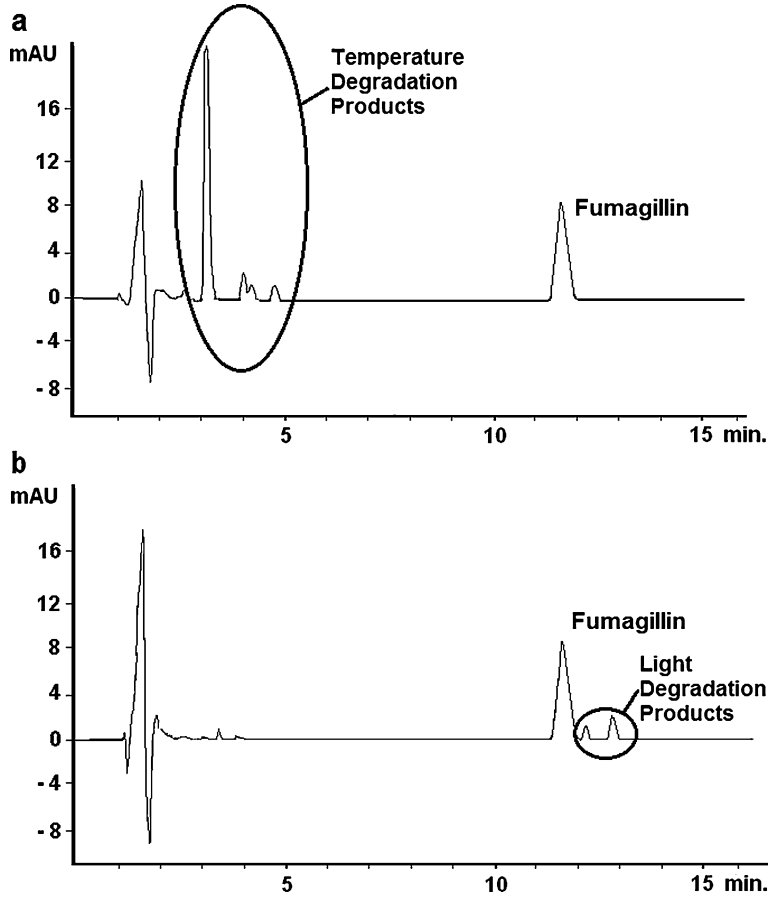

Figure 2. LC-DAD chromatograms obtained at 350 nm from a syrup spiked with 1.5 g of Fumidil B and exposed to **a** temperature and **b** UV radiation.

(Figure 3). The highest dose of 2.5 g, seemed to be somewhat more stable than when the patty was spiked with smaller quantities. Moreover, in amber vials the product was very stable, particularly at higher concentrations, and after 4 weeks the loss was below 10%. In samples stored at 22° C, 30°C and 40°C, the compound was quite stable (Figure 3) and the losses were around 10%.

When samples were exposed to UV radiation, the product was quickly degraded in clear glass vials and after only 3 days almost 75% of the product was degraded (Figure 3). Degradation was slower in amber vials and the rate of loss of this product seemed to be influenced by the concentration of product used. Thus, at higher concentrations the rate of degradation was slower. Curiously, peaks belonging to the degradation products were not observed, probably because the compounds generated were located in an undetectable part of the LC-DAD chromatogram.

3.2 Field trials

In the initial selection of colonies (June 2006), no adult or brood pathogens were detected in any bee colony. At the start of the field treatment (9th October, 2006) all the colonies had been naturally infected by *N. ceranae*, as confirmed in the forager bee samples. *Varroa destructor* was not detected in any sample during the study due to the acaricide treatment, and thus this pathogen could not be associated with the collapse of the

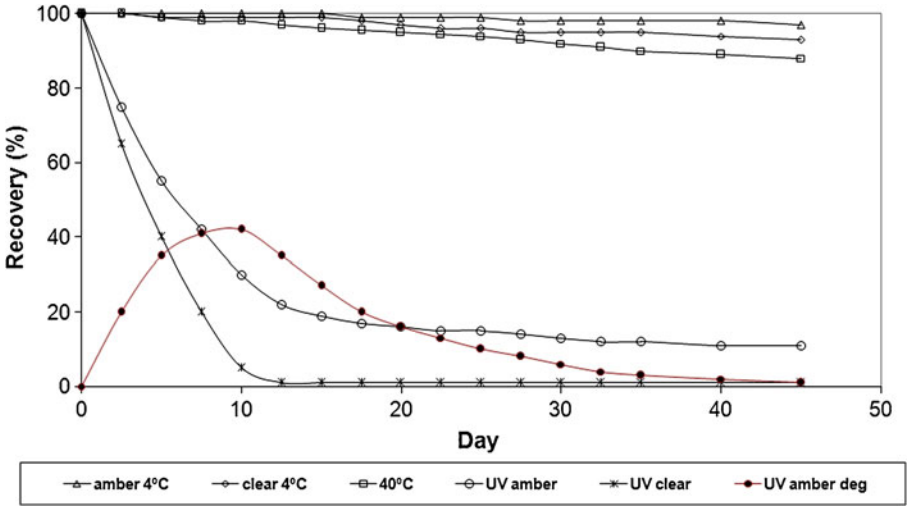

Figure 3. The influence of temperature, UV radiation and container type on the recovery of fumagillin from a mixture honey/powdered sugar spiked with 1.5 g of Fumidil B. The figure shows the data from one aliquot, representing the general tendency of all the samples.

colonies. None of the queens was replaced throughout the study in any colony.

In the colonies that suffered collapse, the only prior sign was that of progressive depopulation leading to the final collapse and death. In such colonies, finally only the queen was found in the beehives, and they were surrounded by about 100–300 young bees and a small amount of brood, along with some stored pollen and honey. There were no other signs observed in adult bees or brood, and there were no bees seen within a 200 m radius around the apiary or dead in the bottom board of the hives.

The efficacy of the different treatments was studied in each colony (summarized in Table II). Only in the 1S group were all the colonies (100%, n=9) free of *N. ceranae* in the first analysis after the end of the treatment (December 15th, 2006) and in this group all the medicated syrup had been consumed by the bees. However, *N. ceranae* was detected in two of the colonies in the 1S group four months after the administration of last dosage. In the 1P, 2P and 2S groups, *N. ceranae* was identified in 66.6% of the colonies on same date but the food had not been completely consumed in some of these colonies. Only 33.3% of the colonies of 2S group, 66.6% of the colonies in the 1P group, and 16.6% of the colonies in the 2P group consumed all the fumagillin administrated. At the end of the test period (13 month p.t.), all the colonies had been reinfected by *N. ceranae*, and in control groups, all the colonies were *N. ceranae* positive throughout the study, producing showing the highest percentage of dead colonies.

There was no colony death in the 1S group at the end of the study and thus, the survival in this group was the highest in relation to the others (Mann–Whitney, $P<0.05$). In the groups used as controls for each dose (C1 plus C2) or in that supplied with pollen substitute (C3), colony death reached about 75% and 80% of the colonies, respectively. In those cases (Figure 4), the survival was always lower than in the groups that received fumagillin treatment (Mann–Whitney, $P<0.05$).

The quantity of food containing fumagillin that was ingested appeared to have a direct influence on the efficacy of the treatment as well as in the final survival of the colonies (CATPCA analysis, $P<0.05$, Figure 5). Indeed, the amount and the type of treatment ingested was closely related and therefore, the effectiveness of the treatment was greatest if the fumagillin was fully consumed. The colonies

Table II. Summarized results of the efficacy of the different treatments and honey bee colony development.

Group	*Nosema ceranae*-positive colonies				*N. ceranae*-positive or dead colonies			
	June 2006	September 2006	October 2006	Colonies that totally consumed the treatment	December 2006	April 2007	December 2007	Total surviving colonies (%)
1S	0	9	9	9	0	2	9	100
2S	0	9	9	3	3	5 1 dead	5 3 dead	55.5
1P	0	12	12	8	4	10 (2)[a] 2 dead	5 (1)[a] 5 dead	41.6
2P	0	18	18	3	7	10 4 dead	10 4 dead	55.5
C1+C2	0	10	10	10	10	2 8 dead	1 1 dead	10
C3	0	12	12	12	12	6 6 dead	2 4 dead	16.6

[a] Queenless

that consumed all the fumagillin were those that were most viable, irrespective of whether the fumagillin was administered in syrup or patty, or of the fumagillin dose (Figure 6). The treatment administered to the 1S group was the most effective and all the colonies of the group consumed all the syrup. The viability of the 1P colonies was lower than those in the 1S group, perhaps since only 66% ingested the whole patty. The colonies in the 2P and 2S groups that consumed all the food with fumagillin displayed a similar viability throughout the study as the

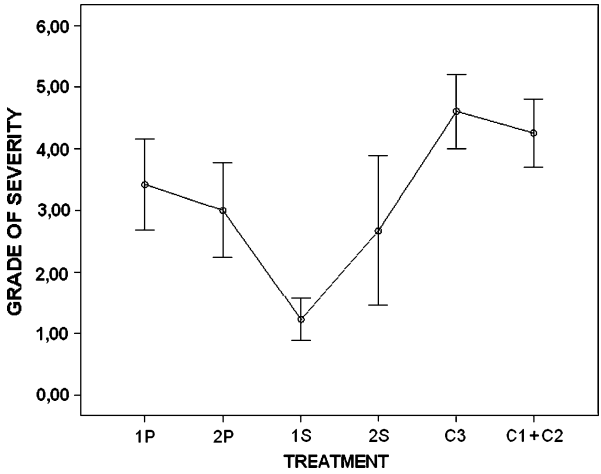

Figure 4. Relationship between infestation and colony survival, irrespective of the treatment applied or consumed: grade 1 (minor severity) corresponds to colonies infected in December 2007 but with no colony death; grade 2 corresponds to colonies infected in April and December 2007 but with no colony death; grade 3 corresponds to colonies infected in December 2006, or April and December 2007, and with no colony death; grade 4 corresponds to infection and colony death in December 2007; and grade 5 (maximum severity) with infection and colony death in April 2007. The analyses were performed with a confidence level of 95%.

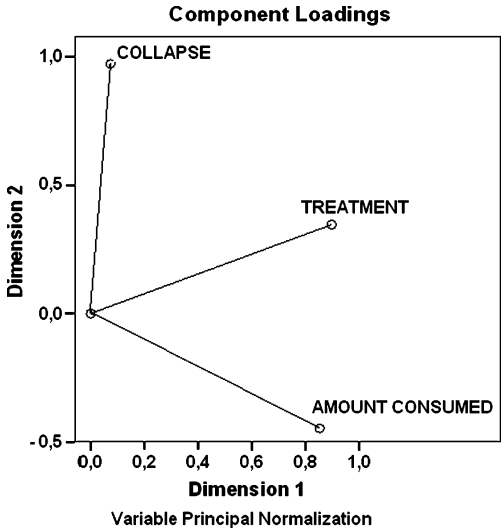

Figure 5. Correlation between the amounts of the medicated preparation consumed, treatment efficacy and the final survival of the colonies (CATPCA analysis).

colonies in the 1S group, which was very different from the viability observed in 1P group (Mann–Whitney, $P<0.05$).

3.3 Fumagillin residues of in honey

When the residues of fumagillin in honey were assessed in all the colonies (January 2007, summarized in Table III), the values ranged from 66 μg/kg to quantities below the limit of detection. The highest residual values found were associated with those groups that received the highest amount of fumagillin (groups 2S and 2P). However, no residues were detected in any of the fumagillin treated colonies in spring and there was no honey production in May 2007 in the control hives and as such, samples could not be collected.

4. DISCUSSION

In this work, we have evaluated the factors affecting the efficacy and stability of fumagillin (as the active ingredient in a commercial product) in the treatment of nosemosis under field conditions.

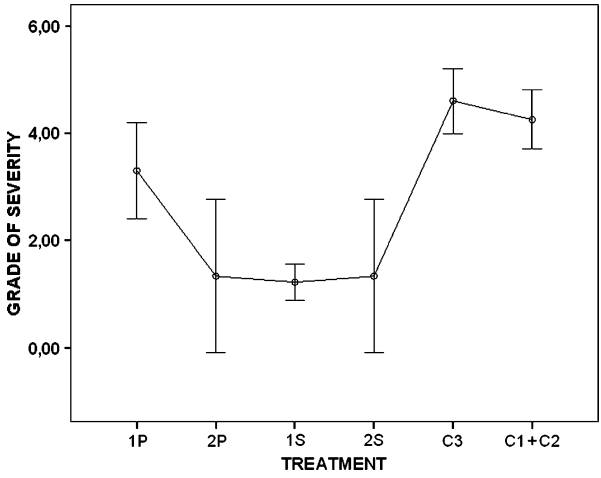

Figure 6. Relationship between infestation and colony survival for the same amount of treatment consumed, whereby the group that consumed all the fumagillin treatment have the same risk: grade 1 corresponded to colonies infected in December 2007 but with no colony death (minor severity); grade 2 corresponded to colonies infected in April and December 2007 but with no colony death; grade 3 corresponded to colonies infected in December 2006, or April and December 2007 and with no colony death; grade 4 corresponded to infection and colony death in December 2007; and grade 5 to infection and colony death in April 2007 (maximum severity). Analyses were performed with a confidence level of 95%.

Table III. Residues of fumagillin in honey from the brood chamber (January 2007) and upper box (May 2007).

Group	Sampling data	
	Jan 2007	May 07
1S	<LOD	<LOD
	<LOQ	<LOD
	15	<LOD
	2	≤LOD
2P	3	<LOD
	17	<LOD
	66	<LOD
	≤LOQ	≤LOD
1P	5	<LOD
	<LOD	<LOD
	<LOD	<LOD
	≤LOD	≤LOD
2S	3	<LOD
	45	<LOD
	54	<LOD
	≤LOD	≤LOD
C1±C2	≤LOD	No honey
C3	≤LOD	No honey

Results in microgrammes per kilogramme of honey
LOD limit of detection, *LOQ* limit of quantification

We have also evaluated its validity as a means of overcoming honey bee colony death associated with *N. ceranae* infection, as suggested previously (Nozal et al. 2008; Higes et al. 2009c).

As a result, we show that fumagillin degradation is clearly affected by the medium (excipient) in which it is applied. Fumagillin was more stable in patty in laboratory conditions, but this was not the most effective means of administration in field conditions, since bees do not ingest the total product. In terms of the appropriate dose, it seems that the effects of storage or of environmental conditions are somewhat more intense on more dilute mixtures. Temperature also affects the final concentration and as the temperature rises, the amount of active ingredient remaining falls. Considering that administration of this product to bees involves it remaining inside the hive, the length of time and the temperature at which the medicated syrup persists inside the colony should be taken into account, especially when performing efficacy tests. Indeed, both these factors together will negatively influence the concentration of the active compound, especially in hot months.

Exposing the preparations to UV radiation produces very rapid degradation of the compound, which is clearly faster in clear glass containers. As a result, the fumagillin peak area decreased considerably and two peaks appear at longer retention times, with another two small peaks observed at shorter times. We believe that these new peaks correspond to neofumagillin diastereoisomers, in accordance with previous observations (Assil and Sporns 1991; Kochansky and Nasr 2004) and mass spectrometry analyses (Nozal et al. 2008). It is noteworthy that when honey–sugar mixtures are irradiated, photodecomposition products are rarely observed despite the notable decrease of the fumagillin peak. Hence, the degradation products are probably located near the peak front. This effect could be due to their polarity, together with the acidic pH of honey, which might favour their more rapid elution. However, although the fumagillin peak area diminishes, other relevant peaks did not appear in the chromatogram, which might explain why the compound was not detected in honey samples from treated beehives in other studies (Assil and Sporns 1991). A slight change in the retention time for the fumagillin peak was also observed, probably due to the high amounts of sugar in the diluted sample injected.

The data presented here show that the quantity of active ingredient in the different presentations, as well as the drug storage and certain environmental conditions can strongly influence the amount of fumagillin final available to the bees. Thus, it is highly recommended that recently prepared solutions of syrup are used (50% water–sugar), which are stored in amber containers and that are kept in the refrigerator. The high rate of fumagillin degradation detected in samples stored in syrup at 22°C, as well as that observed in the more diluted mixtures, must clearly be taken into account when comparing studies on fumagillin treatments. These data indicate that a diluted

mixture of fumagillin maintained in the hive for a long time cannot be considered the correct posology to treat nosemosis.

We have also demonstrated the efficacy of the proposed effective doses of fumagillin against *N. ceranae* infection (group 1S received 1.5 g of Fumidil B in 250 mL of syrup applied four times at weekly intervals). The administration of 250 mL of syrup guaranteed the complete and rapid quick consumption of the product by the bee colony, and each bee probably receives a highly concentrated dose of fumagillin that acts as an effective individual dose. This fact should be also taken into account when comparing the efficacy of different posologies (dosage, frequency of treatment etc.).

In the light of the instability of fumagillin, it is very important to ensure the complete and rapid consumption of the medication to optimise the effectiveness of this treatment. It is also important to ensure that the syrup with fumagillin is correctly prepared (as suggested by Webster 1994), by adequately dissolving the fumagillin and protecting it from the effects of light and heat. *Nosema apis* has been adequately controlled in mating nuclei colonies populated with young worker bees by administering honey–sugar patties containing fumagillin (Gregorc and Sulimanovic 1996). However, fumagillin in honey–sugar patties did not provide the best results in this trial since this preparation was not totally consumed by bees, even though fumagillin was more stable in this medium.

Our work confirms the effectiveness of a fumagillin treatment at a given posology of 1.5 g of Fumidil B in 250 mL of syrup (50% sugar/water) applied four times at weekly intervals to avoid the collapse and death of honey bee colonies infected with *N. ceranae*. Moreover, we were unable to find residues of the active compound in the honey collected, as usually collected for human consumption, under our experimental conditions.

In Canada, different efficacy results were recently obtained when applying 8 L of syrup with a total of 190 mg fumagillin (a very diluted mixture) and only testing the colonies the following spring while establishing intensity by mean spore count (Williams et al. 2010, 2011), a parameter that is quite unreliable to establish the state of the disease (Meana et al. 2010), even when external bees were sampled. However, in this study, neither the quantity consumed, the time needed to consume all the food (medicated or not), nor the conditions of storage were indicated.

Until the product is fully authorized by the EMEA in Europe, adequate treatment in countries with authorization for exceptional use should only be carried out under veterinary control in order to guarantee the correct use and posology of this medication to achieve the best results and avoid the accumulation of residues in honey.

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Stabilité et efficacité de la fumagilline pour contrôler l'infection par *Nosema ceranae* (Microsporidia) chez les abeilles (*Apis mellifera*) en conditions de laboratoire et de terrain.

***Nosema ceranae* / fumagilline / stabilité / sirop / dosage / traitement / dépopulation de la colonie**

Zusammenfassung–Die Stabilität und Effektivität von Fumagillin bei der Behandlung von Infektionen mit *Nosema ceranae* (Microsporidia) bei Honigbienen (*Apis mellifera*) unter Labor- und Freilandbedingungen. Das aus *Aspergillus fumigatus* gewonnene fumagillin ist als einzige Chemikalie zur Behandlung von Infektionen der Honigbiene mit Nosema zugelassen und wird innerhalb der EU als ein Arzneimittel für seltene Krankheiten betrachtet. Ausgehend von der Unsicherheit hinsichtlich der Wirksamkeit von fumagillin bei der Behandlung einer Infektion mit *N. ceranae* wurden in dieser Studie mehrere Faktoren untersucht. Die Stabilität von fumagillin wurde bei verschiedenen Konzentrationen unter Laborbedingungen getestet; dabei wurden der Einfluss verschied-

ener Arzneistoffträger (z.B. Zuckersirup oder –teig), verschiedener Temperaturen und der Einwirkung von UV-Strahlen untersucht. Zusätzlich wurden die Daten eines Feldversuchs an natürlicherweise mit *N. ceranae* infizierten Völkern ausgewertet, die wöchentliche Dosen von fumagillin in Sirup oder Zuckerteig erhielten. Diese wurden 4- bis 8-mal verabreicht, so dass die aufgenommene Gesamtmenge pro Volk bei 6 oder 8 g lag. Der Versuch schloss Kontrollgruppen ein, die Sirup ohne pharmakologische Inhaltstoffe erhielten. Während hohe Temperaturen (30–40°C) fortschreitende Degradation des fumagillins über 20 Tage hinweg verursachten, reduzierte die Einwirkung von UV-Strahlung die ursprüngliche Konzentration bereits innerhalb weniger Stunden drastisch. In der Beimischung zu Zuckerteig und bei Aufbewahrung in gefärbten Glasbehältern war es dagegen sowohl bei Kälte als auch bei Hitze sehr stabil, besonders auch bei höheren Konzentrationen. Nach vier Wochen war weniger als 10% Verlust zu verzeichnen. Obwohl sich in Labortests abzeichnete, dass eine Mischung aus Honig und Puderzucker zur Verabreichung des Medikaments am besten geeignet ist, war die Applikation in Sirup zur Behandlung infizierter Völker in Feldversuchen wirksamer. Die schlechteren Ergebnisse bei Anwendung von fumagillin in Honig–Zuckerteig wurden dadurch verursacht, dass die Bienen das Medikament nicht vollständig aufnahmen. Es ist sehr wichtig, für eine schnelle und vollständige Aufnahme des medikamentenhaltigen Futters zu sorgen um eine möglichst effektive Behandlung zu erreichen, besonders wenn man die Instabilität des fumagillins berücksichtigt. In der Tat scheint die aufgenommene fumagillin-haltige Futtermenge einen direkten Einfluss auf die Wirkung der Behandlung und das Überleben der Völker zu haben. Dementsprechend erschien die Behandlung am effektivsten wenn das Medikament in Sirup verabreicht wurde. Die beste Dosierung bestand aus einer Mischung von 30 mg fumagillin (1.5 g Fumidil B®) mit 250 mL Sirup, die einen Monat lang einmal wöchentlich verabreicht wurde. Nach dieser Behandlung wurden keine Rückstände im während der folgenden 4 Monate produzierten Honig entdeckt, wenn er aus dem Honigraum entnommen wurde.

***Nosema ceranae* / Fumagillin / Stabilität / Sirup / Honig-Zuckerteig / Dosierung / Behandlung / Völkerschwächung**

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