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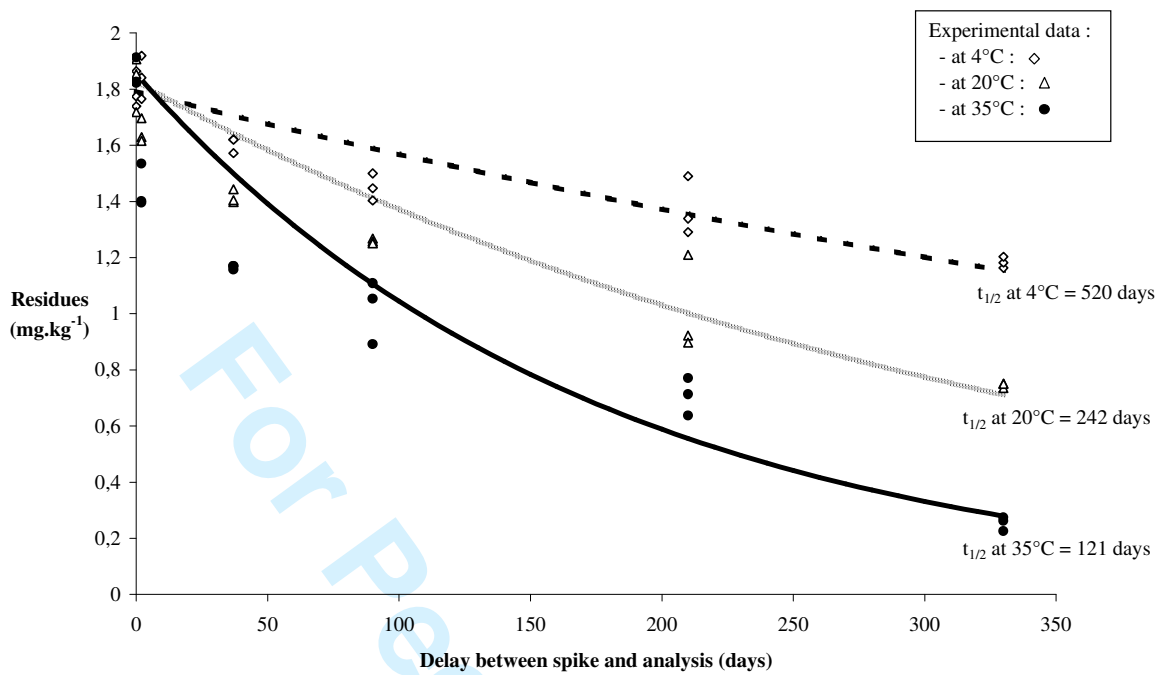


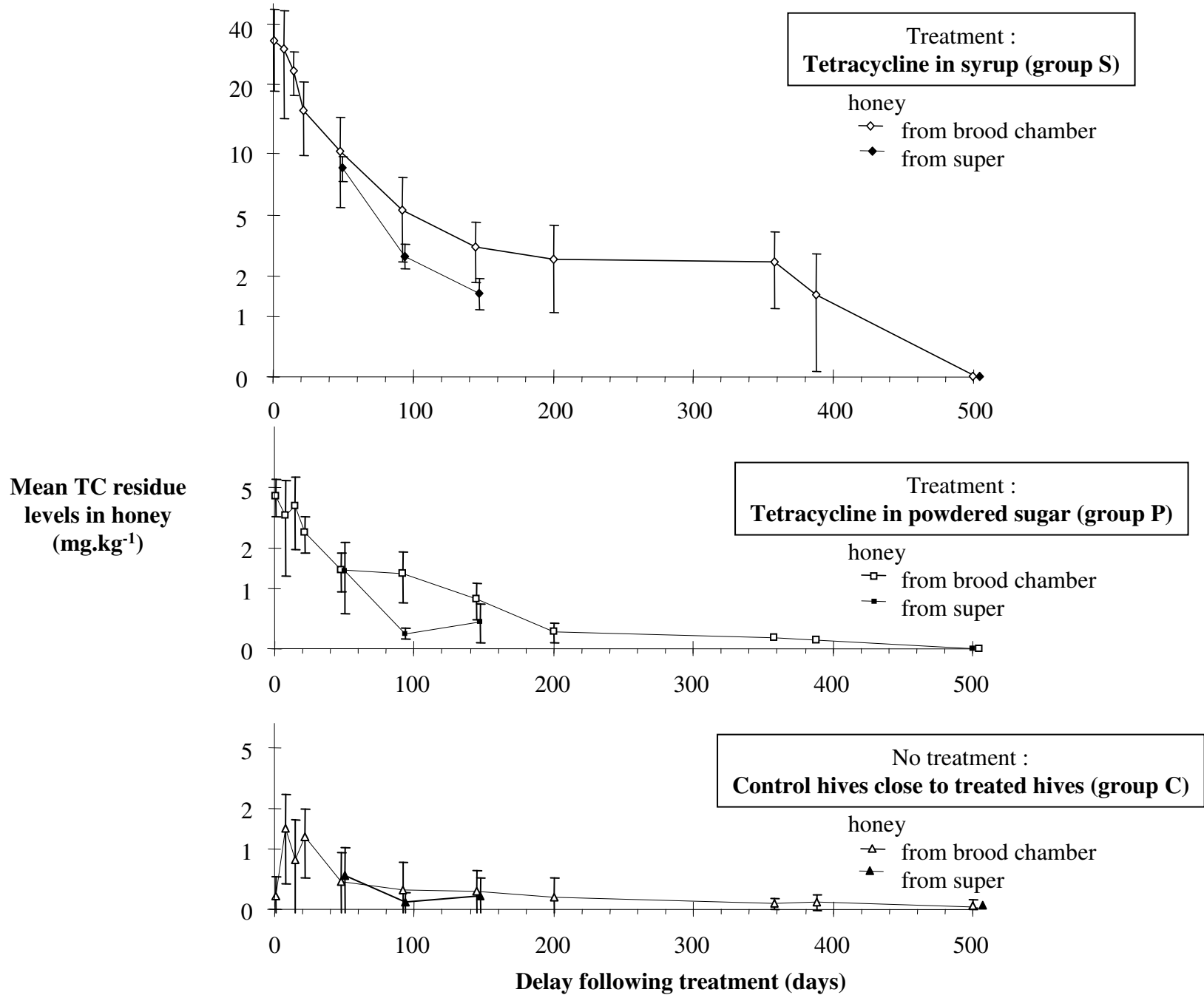
Tetracycline residues in honey after hive treatment

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Table 1. Tetracycline residues in hives

Date of sampling	Sampling	Mean residues (mg.kg ⁻¹)			
		in group DC	in group C	in group P	in group S
T-16	in brood chambers	ND	ND	ND	ND
D+1	in brood chambers	ND	0.16	4.34	<u>40.7</u>
D+8	in brood chambers	ND	1.43	3.32	<u>37.0</u>
D+15	in brood chambers	ND	0.72	3.82	<u>28.8</u>
D+22	in brood chambers	ND	1.22	2.58	<u>18.2</u>
D+33	adding supers in all groups				
D+48	in brood chambers	ND	0.37	1.36	<u>11.2</u>
	in supers	ND	0.45	1.35	9.19
D+92	in brood chambers	ND	0.25	1.27	5.38
	in supers	ND	0.08	0.18	2.82
D+146	in brood chambers	ND	0.23	0.72	3.22
	in honey harvested in supers	ND	0.15	0.35	1.54
D+202	in brood chambers	ND	0.14	0.20	2.72
D+358	in brood chambers	ND	0.06	0.13	2.57
D+390	in brood chambers	ND	0.08	0.10	1.50
	adding supers in all groups				
D+504	in brood chambers	ND	0.03 ^(a)	ND	0.01 ^(c)
	in honey harvested in supers	ND	ND ^(b)	ND	ND

Deleted: 40.67

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T : day of the first application of TC

D : day of the last application of TC

ND : no residue detected

(a) : no residues were detected except in one super with a value of 0.17 mg.kg⁻¹(b) : no residues were detected except in one super with a value of 0.026 mg.kg⁻¹(c) : no residues were detected except in one super with a value of 0.043 mg.kg⁻¹

Table 2. Comparison between tetracycline concentration in brood chambers and in supers

Days after treatment	Determination coefficient (r^2)	Correlation coefficient (r)	Significance of r	Constant term	Significance of the constant term
48	0.92	0.68	P<0.0001 - S	0.36	P=0.32 - NS
92	0.86	0.43	P<0.0001 - S	- 0.13	P=0.44 - NS
146	0.74	0.40	P<0.0001 - S	0.12	P=0.30 - NS
504	0.93	0.14	P<0.0001 - S	- 0.001	P=0.35 - NS

NS : not significant

S : significant

Tetracycline residues in honey after hive treatment

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Abstract

Tetracyclines are used to control bacterial diseases like European and American foulbrood which may cause severe losses in honey bee population and honey production. This study, using 24 hives randomly distributed into four groups of 6 hives, was performed to measure the occurrence of tetracycline (TC) residues in honey following two types of TC application. Two groups of colonies were treated 3 times with 0.5 g of TC in 1 litre of syrup (group S) or in 10 g of powdered sugar (group P). Six hives of a first control group (group C) fed with untreated syrup were installed at 20 and 45 metres from groups S and P respectively. A second control group (group DC) was set up 3 km away. Honey was sampled at different times from all hives and honey artificially contaminated with TC was stored in the laboratory at 4, 20 and 35°C, and all samples were analysed by ELISA and HPLC methods. One day after the last application, the mean TC concentration in brood chamber honey was ten times higher in group S (40.7 mg.kg⁻¹) than in group P (4.34 mg.kg⁻¹). After 8 days, TC residues were detected in all hives of group C. After 146 days, the mean TC concentration in harvested honey was 1.54, 0.35, and 0.15 mg.kg⁻¹ for groups S, P and C respectively. The control group C had been contaminated with TC by drifting. In all hives of the group DC, no residues were detected at any time during the study. The honey collected at day 504 did not contained any detectable TC residues, except in one super from group C (0.026 mg.kg⁻¹). The half-life of TC in honey from supers was similar in groups C, S and P and equal to 65 days. This duration was twice lower than in honey stored in laboratory in similar conditions : at 35°C in the dark ($t_{1/2}$ = 121 days). In honey stored at 20°C, TC was quite stable and its half-life was 242 days. The data from these experiments indicate levels of TC residues in honey after a treatment in hives, their persistence and diffusion into the apiary. These results show that the TC must be used with precaution in honey production.

Keywords : Honey, antibiotics, tetracycline, residues, stability

Introduction

Bacterial diseases of the honey bee *Apis mellifera* may severely decrease the honey bee population and honey production, causing significant damage to the beekeeping industry. Two of most important bacterial diseases that affect the larvae of the honey bee *Apis mellifera* are American foulbrood and European foulbrood caused by *Paenibacillus larvae* and *Melissococcus pluton*, respectively. These pathogens have been treated in bee colonies using antibiotics for many years. Due to their low cost and large availability, tetracyclines are most commonly used in several countries even as a preventive treatment (Evans 2003).

This treatment risks tetracycline residues in honey as reported by many authors. Among them, Faucon *et al.* (2001) found tetracyclines residues in 34 out of 75 French lavender honeys, and 14 of them had residues above 0.020 mg.kg⁻¹. In 2002, residues of tetracyclines (ranging from 0.019 to 2.152 mg.kg⁻¹) were detected in 19 of the 113 French honey samples collected according to the 96/23/EC Directive (National surveillance plan, note DGAL 2002). Only two producers had previously indicated they used tetracyclines (Martel *et al.* 2003).

Usleber *et al.* (1996) showed that seven days after the last treatment of bee colonies with tetracycline (TC) or oxytetracycline (OTC) in sucrose (10 mg.g⁻¹), honey contained more than 0.4 mg of TC/kg and 1.5 to 3.5 mg of OTC/kg. Matsuka *et al.* (1990) showed that only two days after OTC administration, this antibiotic was already distributed in the whole hive (OTC concentration ranged between 15 and 91 mg.kg⁻¹ depending on the proximity to the feeder) and was equalized within one week. Both authors observed that the degradation of OTC in hive was not complete after 6 weeks.

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2 However, currently, no maximum residue limits (MRL) have been defined for tetracyclines and
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4 their metabolites in honey contrary to other matrices such as milk, meat, liver and kidney whose
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6 MRL range between 0.1 and 0.6 mg.kg⁻¹. Switzerland and Belgium have established a tolerance
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8 limit of 0.02 mg of tetracyclines and their metabolites/kg of honey. Above this limit, honey must be
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10 removed from the market. Consequently, the present study was designed to contribute to a better
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12 knowledge of the consequences of antibiotic treatments on their residue levels in honey. Two of the
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14 current protocols used by beekeepers were tested : antibiotics in syrup or in powdered sugar given
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16 to colonies 4 weeks before the honey flow. The decline of TC in hive has been followed in brood
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18 chamber and super honey for 504 days following antibiotic treatment and compared to that in honey
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20 samples stored at different temperatures.
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28 **Experimental**

29 *Materials and reagents*

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31 The homogenisation equipment for TC extraction from honey samples consisted of a Vortex-mixer
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33 with variable speed from Fisher Bioblock Scientific (Illkirch, France) and an agitation system for
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35 centrifuge tubes from New Brunswick Scientific Co. (N.J., USA). The centrifuge GR 4.11 model
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37 from Jouan (Saint-Herblain, France) was used to eliminate pollen and other impurities in honey
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39 samples.
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47 The TC analysis was performed by HPLC on an Agilent 1100 from Agilent (Karlsruhe, Germany)
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49 consisting of a quaternary pump, an autosampler with a 20 µl loop and a photodiode array detector
50
51 (DAD). A second analytical method was based on the ELISA using a Ridascreen® Tetracyclines kit
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53 from R-Biopharm (Darmstadt, Germany) that contains all the reagents required for the assay. Test
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55 kits were stored at 4°C. The detection system for ELISA was a Multiskan Plus MK2
56
57 spectrophotometer from Elvetec Service (Marseille, France).
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1
2 All solvents used for honey sample preparation (acetonitrile, methanol) were ultra pure grade for
3 pesticides analysis and were provided from Fisher Scientific Labosi (Elancourt, France). The *ortho*-
4 phosphoric acid 85% min (RP Normapur for analysis) and Tween 20 were purchased from Merck
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All solvents used for honey sample preparation (acetonitrile, methanol) were ultra pure grade for pesticides analysis and were provided from Fisher Scientific Labosi (Elancourt, France). The *ortho*-phosphoric acid 85% min (RP Normapur for analysis) and Tween 20 were purchased from Merck Eurolab - Prolabo (Fontenay-sous-Bois, France). We used the laboratory-distilled water. All other reagents (NaH₂PO₄, 2 H₂O, Na₂HPO₄, 12 H₂O, and NaCl) were obtained from Fisher Scientific Labosi. The phosphate buffered saline (PBS) was prepared by mixing 12 g NaH₂PO₄, 2 H₂O, 81 g Na₂HPO₄, 12 H₂O and 240 g NaCl with 3 litres of distilled water (pH must be at 7.2-7.4). This concentrated PBS was stored at 4°C for one month and was diluted at 1/10 before use.

Tetracycline hydrochloride (TC) was purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). TC standards solutions were prepared in methanol (100 mg.l⁻¹) and kept at -18°C for one month in dark glass bottles. Just before use, these solutions were diluted to the required concentrations with 0.05 M *ortho*-phosphoric acid or PBS for HPLC or ELISA respectively.

Tetracycline analysis

Two analytical methods were employed to detect TC in honey samples : HPLC and ELISA. At the beginning of the experiment, TC analyses were undertaken by HPLC because residues levels were high. Under the level of 500 µg.kg⁻¹, the quantitative analysis was made by ELISA. According to the manufacturer description, the ELISA test kit recognizes several compounds of the chemical class of tetracyclines, including TC to which this test is reported to display a cross-reactivity of 100%.

HPLC

The procedure for extraction and analysis of TC was based on the Jürgens' (1981) method but was modified as follows. Honey (1 g) was mixed in a beaker with 3 ml of 0.05 M *ortho*-phosphoric acid and transferred to a 10 ml flask. The beaker was then rinsed with 5 ml of 0.05 M *ortho*-phosphoric

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2 acid and the volume of the flask was completed to 10 ml. The extract obtained was ready for HPLC
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4 analysis after filtration on 0.45 μm filter.
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9 The liquid chromatographic column was a Hypersil BDS C18, 5 μm , 250 x 4.0 mm I.D. from
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11 Merck Eurolab-Prolabo. The mobile phase was 0.05 M *ortho*-phosphoric acid/acetonitrile (80:20,
12
13 v/v) at a flow-rate of 1 ml.min⁻¹. Detection of TC was made at the 360 nm wavelength. The
14
15 injection volume was 20 μl and an external calibration was employed. The chromatographic runs
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17 were performed at 15°C. Limits of detection and quantification were 200 and 500 $\mu\text{g.kg}^{-1}$
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19 respectively. Limits of detection (LOD) and quantification (LOQ) were calculated by a signal to
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21 noise ratio of 3:1 and of 10:1 (Directive 91/414/EEC modified by Directive 96/46/CE and
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23 Explanatory Note EEC/844/87, European Union, Brussels).
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30 **ELISA**

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32 Honey (1 g) was agitated using Vortex-mixer with 50 ml of PBS. This extract was subsequently
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34 centrifuged at 3290 g during 30 min at room temperature to eliminate pollen and other impurities.
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36 According to the procedure described for the kit, 50 μl of TC standards prepared in PBS and the
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38 extracts of honey were added to the bottom of each well in duplicate. At the last step, in presence of
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40 TC, the addition of the stop reagent (which contains 1 M sulphuric acid) leads to a colour change
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42 from blue to yellow. The measurement is made by photometry at 450 nm. The absorption is
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44 inversely proportional to the TC concentration in the sample. Limits of detection and quantification
45
46 were 10 and 15 $\mu\text{g.kg}^{-1}$ respectively.
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53 **Protocols**

56 ***Stability of tetracycline in honey stored under laboratory conditions***

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58 The honey used in this study had been harvested in the laboratory apiary at Sophia Antipolis at the
59
60 end of summer 2002 from hives that had never been treated with TC. These hives were kept in a

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2 mixed environment of typical Mediterranean forest (*Quercus suber* and *Q. ilex*) and meadows.
3
4 Therefore, additionally to Mediterranean oaks, sources of pollen, nectar and honey-dew were from a
5
6 large variety of wild plants such as *Dorycnium pentaphyllum*, sages (*Salvia sp.*), thyme (*Thymus*
7
8 *sp.*), rosemary (*Rosmarinus officinalis*), heather (*Erica arborea* and *E. sp.*), arbutus (*Arbutus*
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10 *unedo*), *Inula viscosa*, *Solidago sp.* This honey reflected this polyfloral origin.
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16 Three aliquots of 200 g of honey were spiked each with 4 ml of 100 mg.l⁻¹ of TC solution prepared
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18 in methanol and manually homogenised using a spatula during 10 minutes. These samples with an
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20 initial level of TC equal to 2 mg.kg⁻¹ were then kept in darkness at three different temperatures (4,
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22 20 and 35°C). A fourth honey sample was added with 4 ml of methanol and used as control sample
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24 during analyses. TC analyses were performed on 3 aliquots of each of these samples at day 0, 2, 37,
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26 90, 210 and 330 following this preparation.
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33 ***Tetracycline found in honey following treatments of colonies***

34 Honey bee colonies

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37 Experiments were conducted on 24 10-frame Langstroth new hives purchased at Ickowicz (Bollène,
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39 France) equipped with crown board feeders. Colonies belonged to the small black local race of *Apis*
40
41 *mellifera mellifera* of our laboratory's experimental apiary. They were transferred to new foundation
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43 combs on April 2002. Hives were randomly distributed into four groups of 6 hives. Two treated
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45 groups called 'the syrup' (S) and 'the powdered sugar' (P) and one untreated group called 'the
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47 control' (C) were installed at the same site in the premises of the laboratory at Sophia Antipolis,
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49 Alpes-Maritimes, France. For limiting drifting as much as possible, the distance between hives of
50
51 the treated groups and the distance between treated and control hives (group C) were 10 metres and
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53 20 metres respectively. The fourth group of 6 hives was disposed 3 km apart from the others and
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55 will be called 'the distant control' (DC) in the following lines.
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2 *Varroa destructor* was preventively controlled using Apivar® : two stripes were inserted in the
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4 hives during ten weeks starting in September 2002, in August 2003 and in September 2004.
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6 Additionally, a five-week treatment was initiated in May 2004 following the observation of
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8 parasites. All hives were artificially fed with commercial syrup (2.5 litres/hive of Apiinvert supplied
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10 from Nevières - Valensole, France) in September 2003 and April 2004 and with candy (2.5 kg/hive
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12 of Apifonda from the same supplier) in November 2003 and January 2004.
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16 Colony mortality was observed at day 146 (1 colony of group S), day 202 (2 colonies of group
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18 P), day 358 (1 colony of group P and 1 of group C) and day 504 (1 colony of group S). Similar
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20 mortality rates, colony weakness and a development of wax moth especially in supers were also
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22 observed by other beekeepers in the area and were attributed to the very high temperatures during
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24 summer 2003.
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27 28 29 30 Treatment

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32 Before the start of the experiment, analyses of TC were realised on honey collected in every combs
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34 of all hives 16 days before the first TC application. TC was applied to hives at the end of winter
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36 according to two modes of application : syrup or powdered sugar. Groups S and P received 3
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38 applications of TC (0.5 g each per colony) 7 days apart (on the 26 February, 6 and 12 March 2003).
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40 Group C received saccharose syrup only at the same dates.
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46 Syrup (saccharose/water 50:50 (w/v)) was prepared by mixing 5 kg of saccharose and 5 litres of
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48 water using a magnetic agitator during one night. TC syrup was processed extemporarily by mixing
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50 during 4 hours 3.785 g of TC in 7.5 litres of saccharose syrup. Each day of treatment, each colony
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52 of groups C and S received 1 litre of syrup or TC syrup respectively, precisely measured with a
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54 graduated cylinder.
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2 TC powdered sugar was processed by mixing manually 3.5 g of TC and 70 g of powdered sugar.
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4 Each day of treatment, each colony of group P received 10 g of TC powdered sugar equally dropped
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6 between combs. An aliquot of each mix (saccharose syrup, TC saccharose syrup and TC powdered
7
8 sugar) was kept at 4°C immediately after preparation for TC titration.
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11 12 13 14 Honey sampling in colonies

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16 In every colony, all frames of each brood chamber and super were identified with a number. On day
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18 1, 8, 15, 22, 33, 48, 92, 146, 202, 358, 390 and 504 post treatment, individual samples of 10 g of
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20 honey were taken repetitively from the same five frames in the brood chamber and in the super
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22 from uncapped or sealed honey cells. Honey samples were filtered through a nylon sieve into a
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24 clean polyethylene jar appropriately labelled (identification of the colony, brood chamber or super,
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26 frame, treatment mode, date of sampling). All samples were stored at 4°C before analysis.
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33 Until 22 days after treatments, each sample was analysed individually. After this date, the five
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35 samples from the same colony were pooled before analysis.
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40 Supers were added 33 days (14 April 2003) after the last treatment. On the 5 August 2003 (i.e. 113
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42 days later), honey of each super was harvested separately for group DC, group C, group P and
43
44 group S successively. The extractor was cleaned between each extraction. Honey harvests were
45
46 weighed using an electronic balance (accuracy = +/- 0.05 kg) and were ranged from 15.5 to 18 kg
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48 (mean = 16.5 kg), from 1 to 20 kg (mean = 12.7 kg), from 7.1 to 17 kg (mean = 12.6 kg) and from
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50 11.6 to 16.8 kg (mean = 14.4 kg) for groups DC, C, P and S respectively.
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Data analysis

Degradation of tetracycline in spiked honey stored in laboratory conditions at various temperatures

We assessed that the degradation speed followed a first-order kinetic model according to the equation :

$$k = - \ln(C/C_0)/t \quad (1)$$

where k, the degradation rate is expressed in day⁻¹, C and C₀, the mean TC concentrations (mg.kg⁻¹) of the three samples taken at day t and day 0 respectively. k was estimated by linear regression analysis. The persistence of the TC was estimated by the half-life time (t_{1/2}) using equation 2 :

$$t_{1/2} = \ln 2/k \quad (2)$$

Comparison between the mean tetracycline titre of the five honey samples of the same hives and the tetracycline titre measured on the pooled samples

At day 1, 8, 15 and 22, TC titre was measured independently on each honey sample (one sample per frame) and on the pool of the five samples taken from the same brood chamber. Means of the titres of these five samples and titres of the corresponding pools were compared using the mean square correlation method.

Statistical analyses were realised with the appropriate module of JMP® software (JMP® Design of Experiments, Version 5, 2002, SAS Institute Inc., Cary, NC, USA 27513) and multiple comparisons of means were performed on all pairs of means using Tukey-Kramer honestly significant difference test included in the same software.

Results and discussion

Stability of tetracycline in honey stored under laboratory conditions

All linear adjustments according to the degradation speed model (1) were highly significant which ever was the storage temperature (Prob (theoretical F for 1 and 16 degrees of freedom $> F_{obs}$) < 0.0001). k was evaluated to $1.334 \cdot 10^{-3}$, $2.862 \cdot 10^{-3}$ and $5.737 \cdot 10^{-3} \text{ day}^{-1}$ at 4, 20 and 35°C respectively. Logically, higher storage temperatures resulted in a more rapid degradation of TC : half-life time was evaluated to 520, 242 and 121 days at 4, 20 and 35°C respectively (figure 1). In other words, at 35°C, which is the internal hive temperature, the degradation of TC is twice faster than at ambient temperature (20°C). In our study, the honey had a pH equal to 4.5. As in general, the pH of honey ranges between 3.2 and 4.5, the observed stability of TC is consistent with the known stability of tetracyclines in acidic medium (Chambonnaud 1968, Puyt 1997).

[Insert figure 1 about here]

Tetracycline found in honey following treatments of colonies

No residues of TC were detected in any samples 16 days before treatments (T-16). Treatments with TC were realised at T, T+8 days and T+14 days. Analyses of TC in each aliquot of preparation were realised by HPLC. Values measured for TC in syrup preparations used were 0.51, 0.44 and 0.35 g of TC/l of syrup at day 0, 8 and 14 respectively. The TC values measured in the powdered sugar preparations were 0.38, 0.5 and 0.49 g of TC/g of powdered sugar respectively. Most of these values are very close to the expected titre of 0.5 g of TC/l of syrup and 0.5 g of TC/g of powdered sugar.

Comparison between the mean tetracycline titre of the five honey samples of the same hives and the tetracycline titre measured on the pool of these samples

For groups C, S and P, the correlation between the mean of the individual measures performed on the honey taken from 5 frames and the measure on the pool of these samples is very highly

1
2 significant with $r=1.00$, 0.99 and 0.94 respectively ($P<0.0001$). The slopes of the three correlation
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4 lines are not significantly different from 1 and the intercepts (equal to 0.06 , 0.16 and 0.13
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6 respectively) are not significantly different from 0. These slope and intercept values reveal that the
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8 measure on the pooled samples is a very precise and a non biased estimation of the mean of the
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10 measures of the individual samples. Consequently, after day 22, all titrations have been processed
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12 on pooled samples only.
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15 16 17 18 ***Tetracycline residues in hives*** 19

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21 TC residue levels in the honey samples collected from brood chamber and super are described in
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23 figure 2 and table 1.
24

25
26 [Insert figure 2 about here]
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28 [Insert table 1 about here]
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33 Honey samples from brood chambers

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35 One day after the last application, TC residues were detected in honey from brood chamber of all
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37 hives of groups S and P. Values ranged from 22.7 to 71.0 mg.kg^{-1} (mean = 40.7 mg.kg^{-1}) and from
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39 2.48 to 5.32 mg.kg^{-1} (mean = 4.34 mg.kg^{-1}) for groups S and P respectively. Out of the 6 hives of
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41 the control group C, 3 had TC residues with values ranging from 0.06 to 0.72 mg.kg^{-1} (mean = 0.16
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43 mg.kg^{-1} for the whole group C). The mean residues titre of group S is significantly higher than the
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45 means of groups P and C with no significant difference between these latest groups (Tukey-Kramer
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47 test with α equal to 5%). No TC residues were detected in the group DC.
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54 Several conclusions may be drawn from the TC measurements in honey at day one : (i) the
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56 incorporation of TC in honey is very rapid; (ii) the treatment with syrup entailed a ten times higher
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58 concentration of TC in honey than treatment with powdered sugar. Both results are coherent with
59
60 our observation of the rapid removal of syrup by bees and its storage in cells. As some traces of

1 powdered sugar remained on the top of frames, TC was not so much incorporated in honey than
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3 when TC was given in syrup; (iii) non-treated hives may be contaminated with TC through drifting
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5 from neighbouring TC treated hives. This contamination is related with distance between hives :
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8 whereas no TC residues were detected in the group DC, two of the three contaminated hives of
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10 group C were at the edge of this group i.e. at 20 metres from the first hive of group S. The farthest
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The rapid dissemination of syrup into the whole colony is a well known phenomenon : Courtois *et al.* (1958) demonstrated that 24 hours after application of syrup marked with ^{198}Au , all bees of the hive were radioactive with all the radioactivity detected in the digestive tract. In a similar experiment, Nixon and Ribbands (1952) observed that following application of radioactive syrup to a colony, 43 to 60% of all bees of the colony were radioactive within 27 hours. Whereas, foragers became radioactive faster and at a higher rate (76%) than the rest of the colony, this enhanced their role of TC carrier to other colonies by drifting. All the large larvae in unsealed cells became radioactive within 48 hours. McKee *et al.* (2003) confirmed that OTC treatment reaches larvae rapidly : on the day following a treatment with 0.3 to 1 g of OTC, they measured in larvae an OTC level ranging between 13.3 and 172.2 mg.kg⁻¹.

After 8 days, TC residues were detected in all hives of group C. The TC level decreased significantly in all groups whereas the differences previously observed between groups were maintained. The mean TC level of group S remained significantly higher than those of group P and C until day 358 post treatment. After approximately one year (358 days), mean TC residue levels were 2.57, 0.13 and 0.06 mg.kg⁻¹ for groups S, P and C respectively. At day 390, mean values of TC residues were 1.50, 0.10 and 0.08 mg.kg⁻¹ in groups S, P and C respectively.

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2 No residues were detected after 504 days in 3 hives on 4 for group S, in 2 hives on 3 for group P
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4 and in 4 hives on 5 for group C. At this date (D+504), residue levels detected became weak. It is
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6 worthwhile to note that at this time, the highest value (0.17 mg.kg^{-1}) is measured in group C.
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9 10 11 Honey samples from supers

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13 At day 48 (15 days after adding supers), TC residues were detected in harvested honey in all hives
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15 of groups S and P (except for 2 hives of group S which did not produce any honey). Values ranged
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17 between 7.94 and 10.68 mg.kg^{-1} (mean = 9.19 mg.kg^{-1}) and between 0.61 and 2.58 mg.kg^{-1} (mean =
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19 1.35 mg.kg^{-1}) for groups S and P respectively. TC residues measured in the 3 hives of group C
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21 which produced honey ranged between 0.41 and 1.31 mg.kg^{-1} (mean of the 5 hives of group C =
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23 0.45 mg.kg^{-1}).
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30 At day 92 and 146, the mean TC level of group S was significantly higher than the means of groups
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32 P and C which were not significantly different (Tukey-Kramer test with α equal to 5%). In the
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34 honey harvested at day 146 (113 days after adding supers), TC concentrations had dramatically
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36 decreased. All samples from the treated groups (S and P) contained TC residues with values ranging
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38 from 0.87 to 1.94 mg.kg^{-1} (mean = 1.54 mg.kg^{-1}) and 0.07 to 0.80 mg.kg^{-1} (mean = 0.35 mg.kg^{-1}) in
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40 groups S and P respectively.
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47 In group C, the mean residue level in honey increased as a result of the contamination by drifting of
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49 4 of the 6 hives of the group with concentration ranging from 0.05 and 0.66 mg.kg^{-1} (mean for the 6
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51 hives = 0.15 mg.kg^{-1}). It is worthwhile to note that in 2 hives of group C, the contamination was as
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53 high as the level measured in several hives of group P whereas it did not reached values observed in
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55 group S.
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59 In all hives of the group DC, no residues were detected at any time of the study. As expected, no
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drifting occurred from the treated colonies which were 3 km apart. Each super individually

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2 identified were removed at day 146 (5 August 2003) then replaced on their respective hive at day
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4 390 (5 April 2004). The honey gathered at day 504 (28 July 2004) did not contained any detectable
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6 TC residue, except in one super from group C with a value of 0.026 mg.kg⁻¹.
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11 Data on the correlation between the amount of antibiotics in honey and the doses given as treatment
12 are scarce. Such a correlation has been extrapolated by Douault *et al.* (1974) following the feeding
13 of colonies with 4 litres of saccharose syrup marked with ¹⁴¹Ce : 46 days following the last
14 administration of 1 litre of syrup and 40 days after adding supers, they demonstrated by
15 autoradiography of a frame the flow of marked syrup to supers. The radioactivity in supers ranged
16 between 2.4 and 6.3% of the theoretical residual radioactivity. According to these authors, this
17 indicated that bees had moved 2.0 to 8.4 g of saccharose into every kg of honey gathered in supers.
18 Hypothesising that antibiotics distributed in syrup would follow the same flow as sugar, they
19 concluded that a mean of 0.21% of an hypothetical antibiotic dose should finally be found in honey.
20 This evaluation is seven times lower than our experimental mean value of 1.5% of TC dose found in
21 group S.
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40 Comparison between tetracycline concentration in brood chambers and in supers

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42 In every group, the TC concentration in honey from supers was significantly correlated to the TC
43 concentration in honey from brood chambers measured the same date (table 2).
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46 [Insert table 2 about here]
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49 The correlation coefficient always lower than 1 and the constant term always not significantly
50 different of 0 indicate that the TC concentration in honey from supers was in average always lower
51 than the TC concentration in honey from brood chambers. The correlation coefficient decrease
52 between day 48 and day 146 shows that the TC concentration in honey decreased more rapidly in
53 supers than in brood chambers. This diminution in TC concentration was not due to a simple
54 dilution of TC in the increased quantity of honey as no correlation could be demonstrated between
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2 the mass of honey harvested in the hive and TC concentration of this honey, or between the mass of
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4 honey harvested in the hive and the variation of TC concentration in brood chamber from day 22 to
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6 day 146. The amount of TC harvested with honey can be estimated to a range of 1.1 to 11.3 mg and
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8 13.0 to 29.8 mg for groups P and S respectively which represented 0.07 to 0.75% and 0.9 to 2% of
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10 the TC dose applied in powdered sugar or syrup respectively.
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16 These observations are in accordance with the results of Matsuka *et al.* (1990) who described a
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18 transitory increase in OTC concentration in honey from central combs until 5 to 7 days after
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20 treatment. They shown that this increase was not a result of water condensation in stored honey as
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22 there was no significant increase in sugar concentration but should be due to a new distribution of
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24 the medicated store by bees. After a week or more, they observed no apparent difference between
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26 the central combs and those next to the feeder comb.
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33 Half-life time of TC in honey in brood chambers and supers

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35 Considering the samples from brood chambers, all linear adjustments according to the degradation
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37 speed model (1) were highly significant (Prob (theoretical F for 1 and 9 degrees of freedom > F_{obs})
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39 < 0.0001). Determination coefficient (r²) were 0.89, 0.78 and 0.92 for group C, S and P
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41 respectively. The degradation rate k and the t_{1/2} of TC have been evaluated for the time interval day
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43 1 to day 504, except for group C, for which the time interval was day 22 to day 504, as in this
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45 group, TC level increased until day 22. k was evaluated to 8.0 10⁻³, 12.6 10⁻³ and 12.1 10⁻³ day⁻¹ for
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47 group C, S and P respectively.
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54 We observe that the evaluations of the half-life time of TC in hives varied from 55 to 87 days, and
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56 were lower than the 121 days evaluated for honey stored at 35°C under laboratory conditions.
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58 Considering honey from supers, due to the limited number of measures and to the limited variation
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60 of TC concentration during the period, the calculated half-life time values (77, 60 and 57 days for

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2 group C, S and P respectively) are non significant. Whereas, these values are very close to the $t_{1/2}$
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4 obtained for honey in brood chambers of the same groups.
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9 In summary, TC decline in honey was more rapid in hive than when stored under laboratory
10 conditions at 35°C. Whereas we did not measured the actual temperature in the hive in this
11 experiment, hive temperature is considered to be equal to 35°C and fairly constant due to regulation
12 by bees even if the external temperature is above 38°C (which was the case during summer 2003)
13 (Morse 1990). This raises the question of an active decline of TC by bees.
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22 23 **Conclusion**

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25 Tetracycline application in hives following protocols usually practiced in France with syrup or
26 powdered sugar demonstrated that the antibiotic is very rapidly incorporated into honey. One day
27 after the last TC application, treatment with syrup resulted in a concentration of TC ten times higher
28 than in honey treated with powdered sugar : mean values were 4.34 and 40.7 mg.kg⁻¹ for groups P
29 and S respectively. After approximately one year (358 days), mean TC residue levels in brood
30 chambers were 0.13 and 2.57 mg.kg⁻¹ respectively and after 504 days, residue levels became weak.
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32 Honey collected on day 146 had mean TC levels equal to 0.35 and 1.54 mg.kg⁻¹ in these groups but
33 no detectable residues in honey harvested on day 504. Storage temperature and probably activity of
34 bees influence the half-life time of TC in honey : (i) the degradation of TC in honey kept in jars is
35 twice faster at 35°C compared to 20°C and (ii) the estimated TC half-life time in honey is twice
36 lower in hive ($t_{1/2}$ = 65 days) than when stored under laboratory conditions at 35°C ($t_{1/2}$ = 121 days).
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54 Consequently, the relative stability of TC in honey requires appropriate regulatory measures for
55 asserting honey quality. Either, no honey should be allowed for human consumption following a
56 treatment of the colony with antibiotic during the year, or MRL or tolerance values for antibiotics
57 should be defined. Our data could contribute to this definition.
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4 An important observation is that the honey of non-treated hives may be contaminated with TC
5 through drifting from TC treated neighbouring hives. The contamination occurred from the 1st day
6 until three weeks after treatment and entailed TC concentrations in honey of the same order of
7 magnitude as those measured in some treated hives (mean equal to 0.15 mg.kg⁻¹ in honey harvested
8 on day 146). Moreover, one hive of this control group still contained detectable residues at day 504
9 (0.026 mg.kg⁻¹). This contamination is related with distance between hives : all contaminated hives
10 were located within 20 to 35 metres from the nearest treated hives, whereas no residues were
11 detected in control hives located 3 km apart.
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26 **Acknowledgements**

27
28 We thank Mr B. Delepine and Mr M. Laurentie from AFSSA Fougères for their advice.
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References

- Chambonnaud, J.P., 1968, Contribution à la recherche des antibiotiques dans le miel. *Bulletin Apicole de Documentation Scientifique et Technique et d'Information*, **9**, 133-197.
- Courtois, G., Lecomte, J., 1958, Sur un procédé de marquage des abeilles butineuses au moyen d'un radioisotope. *Comptes-rendus de l'Académie des Sciences.*, **247**, 147-149.
- Douault, P., Louveaux, J., Theurkauff, J., Azoeuf, P., Pintena, J., 1974, Etude du stockage des réserves par les abeilles au moyen d'un sirop de sucre marqué au ^{141}Ce . *Apidologie*, **5**, 107-126.
- Evans, J.D., 2003, Diverse origins of tetracycline resistance in the honey bee bacterial pathogen *Paenibacillus larvae*. *Journal of Invertebrate Pathology*, **83**, 46-50.
- Faucon, J.P., Martel, A.C., Antinelli, J.F., Clément, M.C., Zeggane, S., Cordella, C., Davico, R., Rognone, C., Aurières, C., 2001, Sondage sur la qualité des miels de lavande-lavandin. *Annales de Falsification et d'Expertise Chimiques*, **91**, n°956, 251-261.
- Jürgens, U., 1981, Zur hochdruckflüssigchromatographischen Analyse von Arzneimittelrückständen in Honig. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, **173**, 356-358.
- Martel, A.C., Zeggane, S., Aurières, C. and Davico, R., 2003, Bilan du plan de contrôle 2002 de la contamination des miels, Unité de Pathologie de l'Abeille, AFSSA Sophia Antipolis. 9 p.
- Matsuka, M., Nakamura, J., 1990, Oxytetracycline residues in honey and royal jelly. *Journal of Apicultural Research*, **29**, 112-117.
- McKee, B.A., Goodman, R.D., Saywell, C., Hepworth, G., 2003, Oxytetracycline hydrochloride activity in honey bee larvae (*Apis mellifera*) following medication with various doses. *Apidologie*, **34**, 269-279.
- Morse, R.A. (editor) 1990, *The ABC&XYZ of Bee Culture*, 40th Edition (Medina : A.I. Root Co.)
- Nixon, H.L., Ribbands, C.R., 1952, Food transmission within the honey bee community. *Proceedings of the Royal Society of London*, **140**, 43-50.
- Note DGAL/SDSPA/SDRRCC/N2002-8031 (18 February 2002), according to European Union Directive 96/23/EC, Ministry of Agriculture, Paris.

1
2 Puyt, J.D., 1997, Antibiotiques - Antibiomimétiques. Cours de chimiothérapie anti-infectieuse et
3
4 antiparasitaire, Département de Biologie et Pharmacologie, Ecole Nationale Vétérinaire de Nantes, pp.
5
6 77-90.
7

8
9 Usleber, E., Dietrich, R., Märtlbauer, E., Unglaub, W., 1996, Antimicrobial residues in honey,
10
11 Conference of residues of veterinary drugs in food. *Euroresidue*, Veldhoven, The Netherlands, 948-951.
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Figure 1. Degradation of tetracycline in honey stored in laboratory at 4, 20 and 35°C

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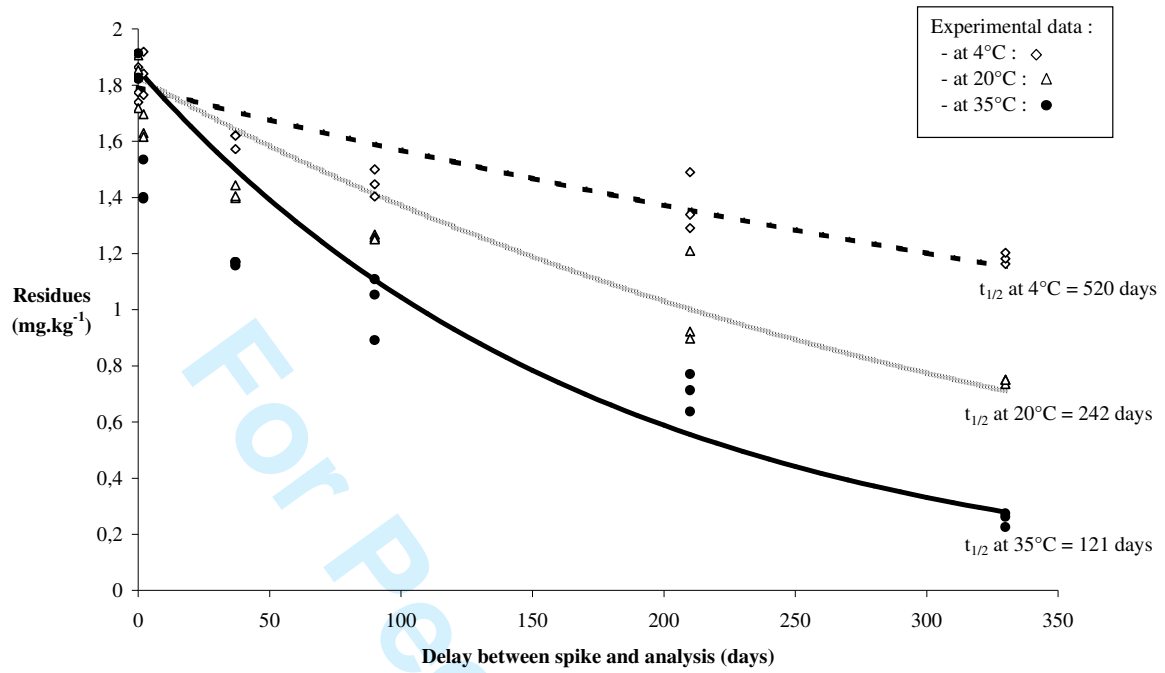


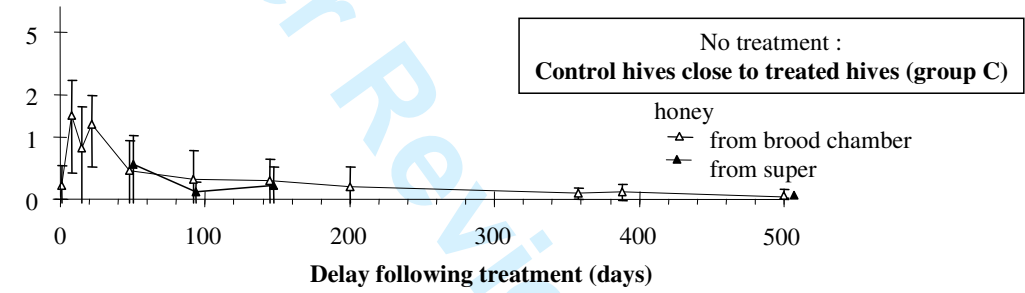
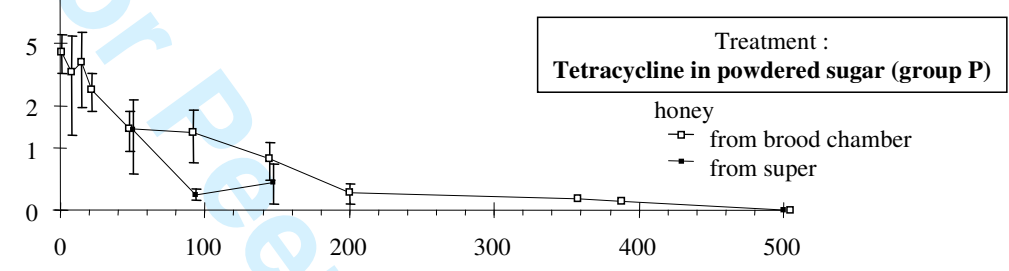
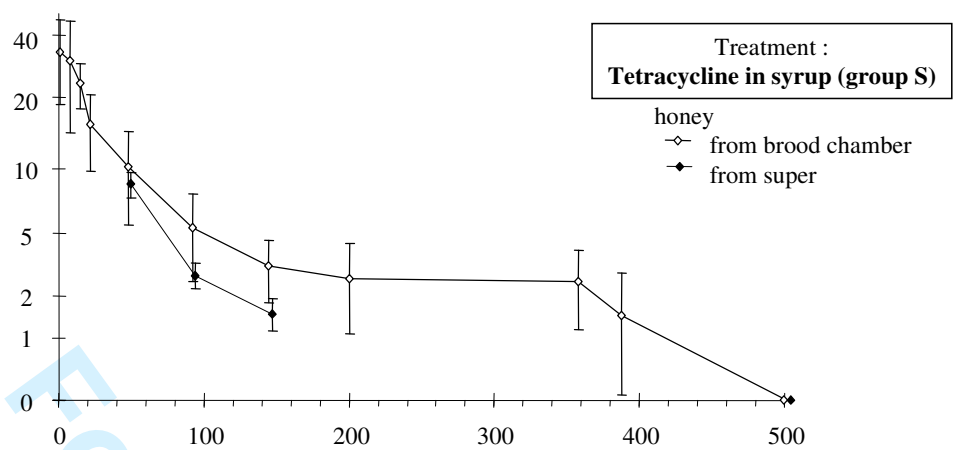
Figure 2. Tetracycline residue levels in honey taken from hives

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Mean TC residue levels in honey (mg.kg⁻¹)



Delay following treatment (days)

Table 1. Tetracycline residues in hives

Date of sampling	Sampling	Mean residues (mg.kg ⁻¹)			
		in group DC	in group C	in group P	in group S
T-16	in brood chambers	ND	ND	ND	ND
D+1	in brood chambers	ND	0.16	4.34	40.7
D+8	in brood chambers	ND	1.43	3.32	37.0
D+15	in brood chambers	ND	0.72	3.82	28.8
D+22	in brood chambers	ND	1.22	2.58	18.2
D+33	adding supers in all groups				
D+48	in brood chambers	ND	0.37	1.36	11.2
	in supers	ND	0.45	1.35	9.19
D+92	in brood chambers	ND	0.25	1.27	5.38
	in supers	ND	0.08	0.18	2.82
D+146	in brood chambers	ND	0.23	0.72	3.22
	in honey harvested in supers	ND	0.15	0.35	1.54
D+202	in brood chambers	ND	0.14	0.20	2.72
D+358	in brood chambers	ND	0.06	0.13	2.57
D+390	in brood chambers	ND	0.08	0.10	1.50
	adding supers in all groups				
D+504	in brood chambers	ND	0.03 ^(a)	ND	0.01 ^(c)
	in honey harvested in supers	ND	ND ^(b)	ND	ND

T : day of the first application of TC

D : day of the last application of TC

ND : no residue detected

(a) : no residues were detected except in one super with a value of 0.17 mg.kg⁻¹

(b) : no residues were detected except in one super with a value of 0.026 mg.kg⁻¹

(c) : no residues were detected except in one super with a value of 0.043 mg.kg⁻¹

Table 2. Comparison between tetracycline concentration in brood chambers and in supers

Days after treatment	Determination coefficient (r^2)	Correlation coefficient (r)	Significance of r	Constant term	Significance of the constant term
48	0.92	0.68	P<0.0001 - S	0.36	P=0.32 - NS
92	0.86	0.43	P<0.0001 - S	- 0.13	P=0.44 - NS
146	0.74	0.40	P<0.0001 - S	0.12	P=0.30 - NS
504	0.93	0.14	P<0.0001 - S	- 0.001	P=0.35 - NS

NS : not significant

S : significant