Apidologie 38 (2007) 534–544
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DOI: 10.1051/apido:2007038

Available online at:
www.apidologie.org

Original article

Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® or Asuntol®50*

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Received 16 March 2007 – Revised 23 August 2007 – Accepted 24 August 2007

Abstract – Acaricide residues were assessed in French commercial beeswax using newly developed liquid and gas chromatography methods. Most of the commercial wax samples and all samples taken during the industrial recycling process contained coumaphos and fluvalinate. Amitraz and coumaphos residue levels were also followed in several hives experimentally treated with Asuntol®50 or Apivar®, two products used in France to control varroa infestation. After the Asuntol®50 treatment, coumaphos residues increased in honey and wax combs, persisted more than 30 days in honey and one year or more in comb wax. The half-life of coumaphos was 69 and 115–346 days in honey and comb wax respectively. Following Apivar® treatment, amitraz was not detected in honey nor in wax. These results are consistent with and complete other studies: the use of coumaphos entails wax contamination which persists through commercial recycling. As this may be a threat for bee health, the use of Asuntol®50 should be avoided.

honey / beeswax / acaricide / residue / contamination

1. INTRODUCTION

Apis mellifera L. is very susceptible to the infestation by *Varroa destructor* Anderson & Trueman, a parasitic mite that affects bees at all stages of development. In Europe, several commercial products are used by beekeepers to control *Varroa destructor*: Apivar® (amitraz), Perizin® or Asuntol®50 (coumaphos), Apistan® (fluvalinate) and Apiguard® (thymol) or Apilife Var® (thymol and other essential oils). In France, Apivar®, Apistan® and Apiguard® are registered for bees. Perizin® was authorized for use on honey bees until 2005 but has never been commercialised, which explains why some beekeepers have used other non-authorized coumaphos formulations, such as Asuntol®50. Apistan® has been widely used but is no longer recommended as the parasite has developed resistance (Faucon et al., 1995; Elzen et al., 1998). The use of pesticides inside beehives implies a risk of contamination of honey and other hive products (beeswax for example) (reviewed by Wallner, 1999). Amitraz, coumaphos and fluvalinate are non-polar and contaminate mostly beeswax (Bogdanov et al., 1998; Wallner, 1999). Following acaricide treatments with Perizin® and Apistan®, brood combs can contain coumaphos and fluvalinate residues ranging from 1.8 to 43.4 mg.kg^{-1} (Bogdanov et al., 1998). Thus, various methods have been developed for determining acaricide residues in wax, especially through the use of gas chromatography (Thrasyvoulou and Pappas, 1988; Van Rillaer and Beernaert, 1989; Lodesani et al., 1992; Bogdanov et al., 1998; Korta et al., 2001).

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Maximal limits of residues in honey were defined in No. 2377/90/EC regulation for amitraz and coumaphos (Regulations): 0.2 and 0.1 mg.kg^{-1} respectively. On the other hand, no maximal limits of residues are fixed for the wax even when it is used for pharmaceutical purposes, food packaging or cosmetics.

In this paper is described an analytical method we have developed to analyse acaricide residues in beeswax samples. We report the frequency of detection of coumaphos and fluvalinate residues in French commercial beeswax. We also analyse wax samples taken at each step of the manufacturing process of comb recycling. Additionally, we designed an experiment to measure the distribution and the decline of amitraz and coumaphos residues in wax and honey from colonies up to one year following Apivar® and Asuntol®50 treatments as both products commonly used in France.

2. MATERIALS AND METHODS

2.1. Materials and reagents

We used a Büchi rotary evaporator equipped with a water bath, an ultrasonic bath and a heating plate, all purchased from Fisher Bioblock Scientific (Illkirch, France). A J2-21M/E centrifuge from Beckman (Roissy, France) and a T25 ultra-turrax blender from Fisher Scientific Labosi (Élancourt, France) were used.

All solvents used (acetone, *n*-hexane, propanol-2, petroleum ether and acetonitrile) were of ultra pure for pesticides analysis grades and were bought from Merck Eurolab - Prolabo (Fontenay-sous-Bois, France). Acetonitrile of HPLC grade was obtained from the same manufacturer. Ammonia (RP Normapur for analysis), sodium chloride, sodium hydroxide, sodium sulfate (reagent grade anhydrous) and florisil 60–100 mesh were purchased from Fisher Scientific Labosi. Florisil 60–100 mesh was heated during 24 hours at 180 °C, cooled down and, after addition of 5% of distilled water (w/w), was stored for a maximum of 4 days. We used the laboratory-distilled water.

Amitraz, coumaphos and fluvalinate were obtained from CIL Cluzeau Info Labo (Sainte-Foy-La-Grande, France). Standard solutions of amitraz, coumaphos and fluvalinate were prepared at a concentration of 100 mg.L^{-1} in acetone and kept at –20 °C for 1 year. The solutions were diluted to the required concentrations with acetone before use and stored at +4 °C for 1 month.

Apivar® and Asuntol®50 used for the apiary experiment were purchased from Véto-Pharma (Château-Thierry, France) and from Bayer-Pharma (Puteaux, France) respectively.

2.2. Acaricides analysis

2.2.1. In honey

The extraction procedure we used has already been described (Martel and Zeggane, 2002). Briefly, twenty grams of honey were mixed in an ultra-turrax blender with a mixture of *n*-hexane (60 mL) and propanol-2 (30 mL) with 0.28% of ammonia (pH = 8). The solution was filtrated through a paper filter and the same operation was realized once. The ultra-turrax was rinsed with 40 mL of *n*-hexane. The combined extracts were transferred to a separating funnel (500 mL). Distilled water with 0.28% of ammonia (50 mL) was added. The aqueous phase (inferior) was discarded and the operation was repeated twice with 50 mL of basic distilled water (pH = 10). The *n*-hexane phase was filtered through a layer of anhydrous sodium sulfate (ca. 10 g) placed in a funnel plugged with a paper filter. The combined extract was concentrated by evaporation to dryness under reduced pressure in rotary evaporator using a 35–40 °C water bath. The residue obtained was dissolved in 1 mL of acetone and was ready for HPLC analysis after filtration on 0.45 μm nylon filter.

Liquid chromatography (HPLC) was performed on a Hewlett Packard 1100 from Agilent (Karlsruhe, Germany) consisting of a rheodyne model 7725 injector with a 20 μL loop and with a photodiode array detector (DAD). The liquid chromatographic column was a Lichrospher 100 RP-18, 5 μm, 250 × 4.0 mm I.D. from Merck Eurolab - Prolabo. The mobile phase (pH = 9) was acetonitrile/water 80:20 (v/v) with 0.28% ammonia at a flow-rate of 1 mL.min^{-1}. The wavelengths used for amitraz and coumaphos were 289 nm and 313 nm respectively.

2.2.2. In wax

Two separate procedures were used to extract pesticides from the wax combs: one for amitraz and fluvalinate and one for coumaphos.

2.2.2.1. Amitraz and fluvalinate

For amitraz and fluvalinate extraction, 3 g of sample was weighed into an erlen (100 mL), extracted with 15 mL of petroleum ether and 10 mL of sodium hydroxide 0.1 M. The extract was warmed at approximately 50 °C during 15 min and the extract was agitated frequently. After cooling, add 4 × 30 mL of acetonitrile saturated with petroleum ether. The solid/liquid mix was agitated and was transferred in a funnel (1000 mL) after filtration on quartz of wool. In the funnel (1000 mL), 700 mL of distilled water saturated with chloride sodium (2%) and 100 mL of petroleum ether were added. The separating funnel was shaken vigorously and the filtrate was allowed to separate into two phases. The inferior phase was collected in other funnel (1000 mL) and was extracted once again with 100 mL of petroleum ether. The superior phase was combined with the first one and the combined extract was extracted in the funnel with 200 mL of distilled water saturated with chloride sodium (2%). The separating funnel was shaken vigorously and the filtrate was allowed to separate into two phases. The inferior phase was eliminated and the superior phase was collected and filtrated through anhydrous Na_2SO_4 into a 500 mL round-bottom flask. The combined extract was concentrated by evaporation to dryness under reduced pressure in rotary evaporator using a 35–40 °C water-bath. The residue was recovered with 1 mL of acetone and was ready for HPLC analysis after filtration on 0.45 μm nylon filter. The liquid chromatographic parameters were similar to the ones for the honey. The detection was realized at 289 nm and 254 nm for amitraz and fluvalinate respectively.

2.2.2.2. Coumaphos

The procedure for extraction and analysis of coumaphos was essentially as described by Bogdanov et al. (1998) but was modified as follows. One gram of sample is weighed into a centrifugation tube, extracted with 10 mL of *n*−hexane in an ultrasonic-bath until complete dissolution of the wax. The sample was frozen (during 2 hours) and centrifuged 20 min at 15000 t/min and –10 °C. The *n*−hexane supernatant was refrozen and centrifuged to eliminate fat contents. The *n*−hexane supernatant was then purified on a florisil column. The glass column (30 cm long glass column and 0.5 cm ID) was filled with 0.1 g of anhydrous sodium sulfate and 1.5 g of florisil. 10 mL conditioning solvent (*n*−hexane) was passed through the column. 5 mL of the supernatant were poured into the column, washed with 20 mL of *n*−hexane and eluted with 20 mL acetone/*n*−hexane 60:40 (v/v). The extract was evaporated to dryness and dissolved in 1 mL of acetone and was ready for GC analysis after filtration on 0.45 μm nylon filter.

The analysis of coumaphos in beeswax was carried out by capillary gas chromatography using the Perkin Elmer AutoSystem XL gas chromatograph (Perkin Elmer, USA), equipped with a nitrogen-phosphorus detector (temperature, 280 °C), a split/splitless injector (temperature, 250 °C) and an autosampler. The gas chromatographic column was a PE-XLB from Perkin Elmer (Courtabœuf, France), 30 m × 0.25 mm (I.D.), 0.25 μm film thickness. The carrier gas was helium 55 (2 mL/min) and the injection volume was 3 μL (split mode). The GC program applied was: 125 °C for 10 min, 275 °C at 4 °C/min for 10 min, for coumaphos analysis.

2.3. Commercial beeswax samples

Forty-seven beeswax samples were obtained from two French apicultural manufacturers and were analysed for an array of acaricides residues. Then, other beeswax samples were collected from one factory to assess coumaphos and fluvalinate residues before and after wax processing. In this factory, the wax originating from old combs provided by different beekeepers is pooled in 500–1000 kg containers and heated at 85–90 °C for 2 hours. Samples for coumaphos and fluvalinate residue analysis were taken at two steps: in liquid mix and from foundation wax produced from the sampled mixes (three different samples of mix and three samples per sampled mix).

2.4. Experimental acaricide treatment of colonies

2.4.1. Honey bee colonies

Experiments were conducted on colonies maintained in Langstroth hives newly purchased at Ickowicz (Bollène, France). Hives were equipped with crown board feeders and with 10 frames from two origins: 5 never used frames with foundation wax from the same manufacturer (‘honeycombs’) and 5 frames with built combs and their colony from a professional beekeeper (‘brood combs’). Following the usual apicultural practice, built combs were

placed in the centre of the brood chamber and new frames with foundation wax at both sides. Bees belonged to the race of *Apis mellifera mellifera.* Hives were randomly distributed into two groups of 5 and installed in the premises of our laboratory. The distance between the two treated groups ('Apivar' and 'Asuntol') were 20 metres.

No significant mortality was observed during the experimental period, except one colony from the 'Asuntol' group, whose queen began laying eggs that gave rise to only drones one year after the beginning of the experiment.

2.4.2. Treatment

Acaricides treatments were applied in April 2001. The five hives of group 'Apivar' received two Apivar® strips during 10 weeks and the five of group 'Asuntol' were treated twice at a 7 day intervals with 10 g of preparation of Asuntol®50 (mix of 15 g of Asuntol®50 and 500 g of powdered sugar) dropped in equal amounts between the different combs. The Asuntol®50 treatment was repeated in March of the following year. Days of treatment were counted as day 0, 7, 347 and 354.

2.4.3. Sampling in colonies

On the day before the first acaricides application, wax and honey were sampled in every combs of all hives for acaricide analysis.

On day 1, 7, 8, 14, 33, 347, 372 and 495, individual samples of wax (both from honeycomb and brood comb) were taken repetitively from the same individual combs, located in the brood chamber and super of each hive. About 10 cm^2 of wax were scraped down to the foundations at the centre and at the periphery of the comb. Wax samples were filtered through a nylon sieve into a clean polyethylene jar appropriately labelled (identification of the colony and frame, treatment group, date of sampling) and kept at –80 °C before analysis. Honey samples obtained by wax filtration were stored at 4 °C until analysis.

2.5. Statistical analyses

Statistical analyses were realized with the appropriate module of JMP® software (JMP® Design of Experiments, Version 5, 2002, SAS Institute Inc., Cary, NC, USA). Classical analysis of variance was used for comparing the residue levels in wax batches. The data obtained on the same hives repeatedly across time were compared using the multivariate model for longitudinal data. For this purpose, the transformed variable log (residues +1) was used to minimise the correlation between the mean and the variance of the experimental data.

We assessed that the degradation speed of amitraz and coumaphos 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 C_0, the mean acaricide concentrations ($mg.kg^{-1}$) of the five samples taken at day t and day 0 respectively. k was estimated by linear regression analysis. The persistence of amitraz and coumaphos was estimated by the half-life time ($t_{1/2}$) using equation (2):

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

3. RESULTS

3.1. Chemical analysis

The separation of coumaphos and amitraz in wax by gas and liquid chromatography respectively is presented in Figure 1.

Calibration curves were constructed from peak areas versus acaricide concentrations. Good linearity was observed (r >/= 0.995) for the studied molecules. The determination of acaricides was achieved by using the external standard method.

Limits of detection (LOD) and quantification (LOQ) were calculated by a signal-to-noise ratio of 3:1 and 10:1 (Directive). The LOQs of the different pesticide were determined after spiking wax and honey samples at lower concentration levels. The LOD and LOQ for acaricides in the two matrices are presented in Table I.

3.2. Residues of coumaphos and fluvalinate in commercial beeswax

Residues of coumaphos and fluvalinate were detected in wax samples provided by the manufacturers. Coumaphos was detected

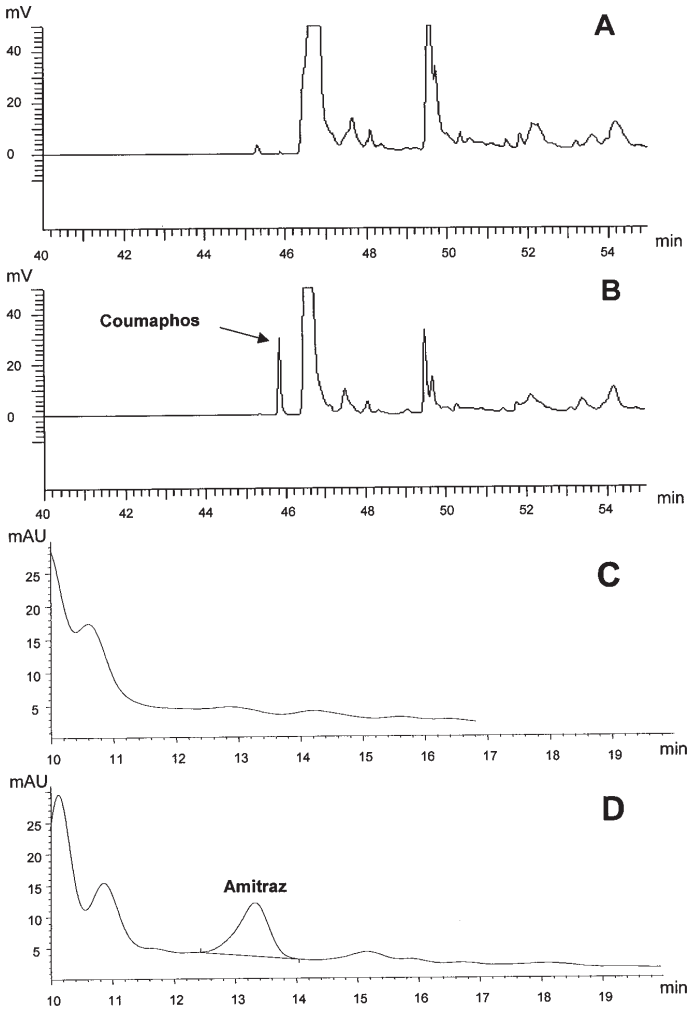

Figure 1. Gas chromatography: **(A)** Chromatogram of a blank extract of wax with a nitrogen-phosphorus detector; **(B)** Chromatogram of wax spiked with 1 mg.kg^{-1} of coumaphos; Liquid chromatography: **(C)** Chromatogram of a blank extract of wax at 289 nm; **(D)** Chromatogram of wax spiked with 1 mg.kg^{-1} of amitraz.

Table I. LOD and LOQ for amitraz, coumaphos and fluvalinate in wax and honey.

Acaricide	Amitraz		Coumaphos		Fluvalinate	
Matrix	wax	honey	wax	honey	wax	honey
LOD (mg.kg^{-1})	0.01	0.002	0.08	0.005	0.03	0.003
LOQ (mg.kg^{-1})	0.03	0.005	0.26	0.015	0.1	0.01

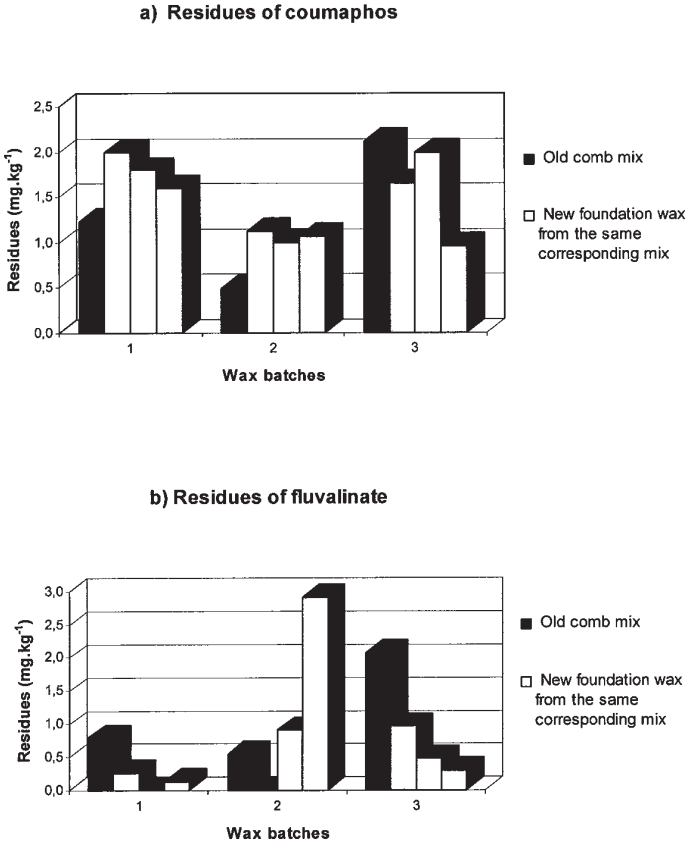

Figure 2. Residues of coumaphos and fluvalinate in commercial wax: in the mix prepared with old combs and in the three samples of foundation wax issued from the mix.

in 42 out of 47 wax samples with values ranging from 0.27 to 5.81 mg.kg^{-1} and fluvalinate was detected in 33 out of 47 wax samples with values ranging from 0.13 to 3.62 mg.kg^{-1}. Both compounds were also detected in the wax mixes (values ranging from 0.49 to 2.12 mg.kg^{-1} and from 0.54 to 2.07 mg.kg^{-1} for coumaphos and fluvalinate respectively) and in the three samples of foundation wax produced from the same mixes (values ranging from 0.96 to 2 mg.kg^{-1} and from 0.13 to 2.91 mg.kg^{-1} for coumaphos and fluvalinate respectively) (Fig. 2). There is no significant difference in coumaphos residue levels among the three sets of foundation wax ($F = 3.87$ with 2 and 6 degrees of freedom (d.f.)) and logically, no significant correlation of these values with the coumaphos residue level in the mix of origin. The conclusions are the same for fluvalinate residues ($F = 1.26$ with 2 and 6 d.f.).

3.3. Residues following Apivar® and Asuntol®50 treatments

3.3.1. In honey

After Apivar® treatment, no residue of amitraz was detected in honey, regardless of the date of sampling. Coumaphos was not detected in honey collected before Asuntol®50 applications. After the two applications of coumaphos in hives, residues reached a mean level of 2.02 mg.kg^{-1} in honey sampled in the brood chambers and were detected up to 26 days

Table II. Coumaphos residues in honey after Asuntol®50 treatment.

Date of sampling (Days)	Sampling	Mean residues (mg.kg^{-1})
D-1	in brood chambers	ND
	D: first application of Asuntol®50	
D+1	in brood chambers	0.23
D+7	in brood chambers	0.69
	D+7: second application of Asuntol®50	
D+8	in brood chambers	2.02
D+14	in brood chambers	1.44
D+33	in brood chambers	0.05
	no honey was harvested from the supers due to the scarcity of honey production	
D+347	in brood chambers	0.02
	D + 347: third application of Asuntol®50	
	D + 354: fourth application of Asuntol®50	
D+372	in brood chambers	ND
D+495	in brood chambers	ND
	in honey harvested in supers	ND

ND: no residue detected.

after the second application of coumaphos (Tab. II).

3.3.2. In wax

Residues of amitraz were detected one day after treatment with Apivar® in the centre of the two combs that were closest to the strips on both sides. The residue values in the brood combs ranged between 0.07 and 2.35 mg.kg^{-1}. On the other hand, no residue was detected in the periphery of the comb and neither in beeswax beyond one day following application.

Before applications of Asuntol®50, coumaphos was present in all wax samples taken from brood combs (mean levels = 2.27 and 3.78 mg.kg^{-1} for central and peripheral parts of brood combs respectively) (Fig. 3A, B). No residue of coumaphos was detected in the centre of honeycombs contrary to the periphery of honeycombs (mean level = 0.17 mg.kg^{-1}) (Fig. 3C, D). On the day following the first application of Asuntol®50 in spring, the mean residual concentrations increased up to 13.9–15.1 mg.kg^{-1} and to 6.78–12.5 mg.kg^{-1} for honeycombs and brood combs respectively. Seven days after this first application, coumaphos concentration decreased by 12 and 58% on average in the centre and peripheral of brood combs respectively. One day after the second application, the mean residual concentration increased again. All these variations are statistically highly significant whereas there is neither significant relation with the comb type (honey or brood) nor with the area sampled on the combs (centre or periphery)[1]. Approximately one year (340 days) after the spring treatment, residues were still detected in the brood chambers despite a significant decrease[2]. The second treatment (on days 347 and 354) entailed a new highly significant increase[3]. Five months after the second treatment (488 days after the first one), the mean concentrations of coumaphos in the brood chambers significantly decreased but still ranged from 3.36 to 3.88 mg.kg^{-1} in honeycombs (Fig. 3C, D) and from 1.61 to 4.71 mg.kg^{-1} in brood combs (Fig. 3A, B) for central and peripheral parts respectively[4]. No residue was detected in combs from the supers except in the peripheral part of two combs, which contained traces and 0.34 mg.kg^{-1} of coumaphos.

[1] The statistics for these three temporal variations are $F_{1/17} = 8.7$; $F_{1/17} = 10.8$; $F_{1/15} = 8.2$ and $P = 0.0005$; 0.0043; 0.015 respectively.

[2] The statistics are $F_{1/12} = 8.2$ and $P = 0.014$.

[3] The statistics are $F_{1/13} = 10.8$ and $P = 0.006$.

[4] The statistics are $F_{1/13} = 4.8$ and $P = 0.047$.

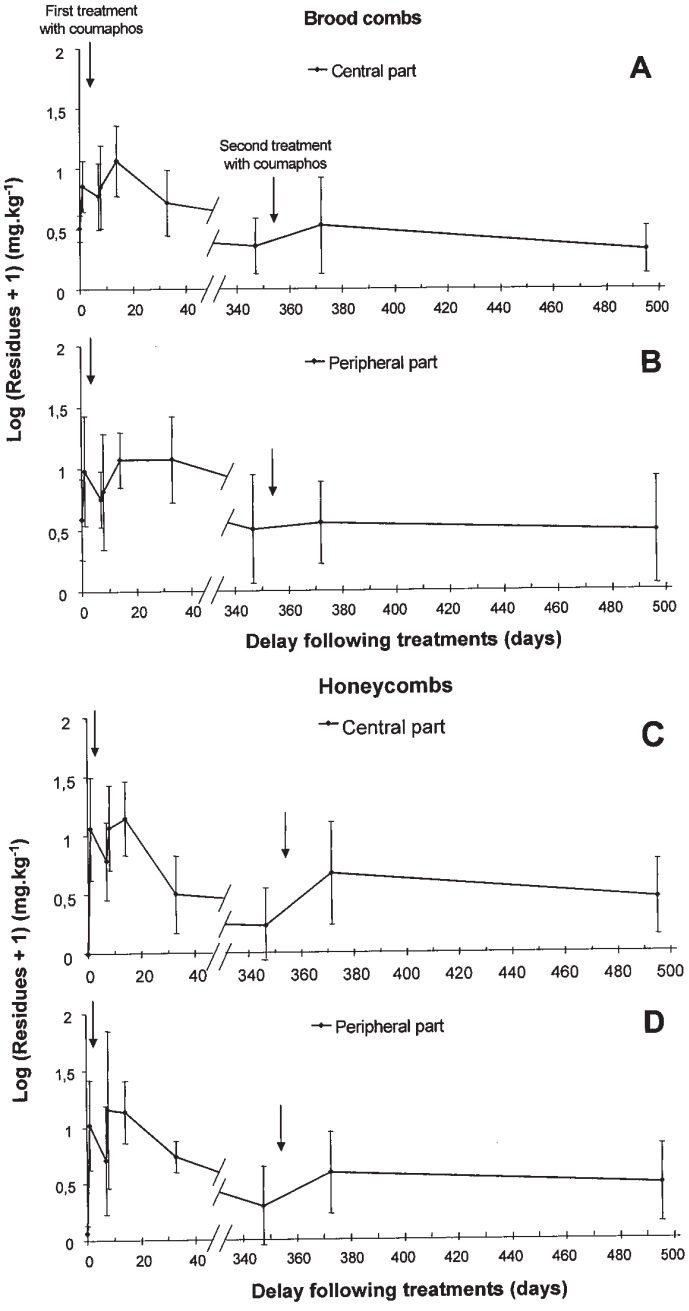

Figure 3. Residues of coumaphos measured at the centre (A) and the periphery (B) of already built combs and at the centre (C) and at the periphery (D) of frames newly equipped with foundation wax.

4. DISCUSSION

4.1. Acaricide residues in commercial waxes

Residues levels measured in foundation wax from old combs indicate that coumaphos and fluvalinate were not degraded despite heating during manufacturing. This is consistent with Bogdanov et al. (1998) who observed that coumaphos contents did not decrease in wax after 2 hours at 140 °C.

The lack of correlation between the coumaphos or fluvalinate concentration in the new recycled wax and the mix from old combs may result from the lack of homogeneity of the mix. The large containers (500 to 1000 kg capacity) in which old combs of various origins are melted are not equipped with any agitator system. Moreover, the content of the container is never totally drained, thus previously melted combs can contaminate the new mixes.

4.2. Residues in honey from experimental apiary

4.2.1. Following treatment with Apivar®

The lack of residue in honey is related to the instability of amitraz in an acidic medium (Berzas Nevado et al., 1990). Amitraz was nearly completely degraded within 10 days in honey (Korta et al., 2001). In fortified honey stored in the laboratory at 40 °C, Martel and Zeggane (2002) showed that no residue was detected after 3 days.

4.2.2. Following treatment with Asuntol®50

Coumaphos diffuses from wax into honey in high proportions compared to other varroacides (Wallner, 1992). Kochansky et al. (2001) observed that after 26 weeks of contact with wax containing 100 $mg.kg^{-1}$ of coumaphos, concentration in honey reached 0.037 $mg.kg^{-1}$. In our study, residues of coumaphos in honey reached levels under the MRL (0.1 $mg.kg^{-1}$) 26 days after the last application of Asuntol®50. The half-life of coumaphos in honey was estimated to be 69 days. Korta et al. (2001) observed coumaphos stability in honey for about 9 months and Thrasyvoulou and Pappas (1988) indicated that the residues of coumaphos in honey decrease rapidly during the first month and more slowly afterwards. Thus, Tremolada et al. (2004) observed that in both media (honey and wax), the coumaphos concentration increases during one month following treatment, seems to level off and then starts to decrease slowly.

4.3. Residues in wax from experimental apiary

4.3.1. Following treatment with Apivar®

Residues of amitraz found one day after the application of the strips (1 g of amitraz applied per hive) were due to the direct contact of wax and strips. No residue of amitraz was detected in wax beyond one day after treatment.

Amitraz is fat-soluble, volatile and very instable in wax (Wallner, 1999; Korta et al., 2001). It was nearly completely degraded within one day in beeswax (half-time = 6.3 hours) (Korta et al., 2001).

4.3.2. Following treatment with Asuntol®50

The treatment with Asuntol®50 powder poured between the combs consisted of an application of 300 mg of coumaphos per hive (4.7 times higher than the quantity of coumaphos administrated with Perizin solution). This treatment entails residues with a concentration 4 to 15 times higher in wax than in honey 24 hours after the last application. All results reveal a large variation between hives. This may be related to bee activity. The Asuntol®50 powder that covers bee bodies is distributed throughout the colony by the bee's movements. Van Buren et al. (1992) indicated that honey bees fix varroacides, such as coumaphos, in their fat tissues and on their body surface. By tagging parts of wax with radioactive tracers, Darchen (1980) showed that

all combs of the hive from brood chambers and supers were radioactive within 24 hours, with a gradient in relation to the distance of the marked frame. The author observed a lower radioactivity in wax from supers (except for the frame above the marked frame), which is consistent with the lower residues of coumaphos we measured in wax from supers. After approximately one year, the brood chambers contained residue levels of 10 to 30 $\mu g.kg^{-1}$ for honey and of 0.8 to 13.8 $mg.kg^{-1}$ for wax. Wax acts as a sink for hydrophobic substances such as coumaphos (Tremolada et al., 2004). Van Buren et al. (1992) observed that wax from colonies that had not been treated with Perizin® for 6 months and up to 18 months did still contain coumaphos (7 and 1 $mg.kg^{-1}$ respectively). Wax newly produced by bees may contain coumaphos residues because there might be an exchange of coumaphos between the combs and the bee's cuticle, which contains hydrocarbons (Van Buren et al., 1992). Consequently, larvae and bees that are exposed to coumaphos demonstrated low but relatively constant concentrations within the range of 1 to 30 $\mu g.kg^{-1}$ in larvae (Tremolada et al., 2004). These residues may be a threat for honey bee. This is confirmed by Pettis et al. (2004) who observed that wax residues can adversely affect at least queen rearing.

In conclusion, the contamination of the French commercial beeswax by coumaphos originates from the use of various non-authorized preparations against *Varroa destructor*. Old combs from all origins arriving to the manufacturers are not systematically tested for coumaphos and are mixed. Furthermore, the coumaphos is not degraded by the heat treatment of wax. Following a treatment with Asuntol®50, coumaphos is very rapidly incorporated in wax mainly and honey. The half life of coumaphos in wax from the honeycombs is 115 days. In wax from the brood combs, coumaphos seems more persistent in the periphery than in the centre of the combs ($t_{1/2}$ = 346 and 138 days respectively). Five months after a second treatment with Asuntol®50, the mean residues of coumaphos ranged from 1.6 to 4.7 $mg.kg^{-1}$ in the wax of the brood chambers and no or weak detectable residues were observed in the supers. In the honey from the brood chambers and supers, no residue of coumaphos was detected at the same delay. After a treatment with Apivar®, no residue of amitraz was detected in wax and honey.

Consequently, as the presence of coumaphos residues in hive may pose a problem for larvae and adult bees, the use of Asuntol®50 should be avoided.

ACKNOWLEDGEMENTS

We thank the two wax manufacturers to their participation in this study. This study was realized in the frame of the EEC apicultural program (FEOGA project) and we thank the European Commission for the financial support.

Résidus d'acaricides dans le miel et la cire après traitement des colonies d'abeilles à l'Apivar® ou à l'Asuntol®50.

miel / cire abeille / résidu / acaricide / contamination

Zusammenfassung – Akarizid-Rückstände in Honig und Bienenwachs nach der Behandlung von Bienenvölkern mit Apivar® oder Asuntol®50. Zur Bekämpfung der Varroamilbe werden in Frankreich die Akarizide Amitraz, Fluvalinat und Coumaphos eingesetzt. Offiziell zugelassen ist dafür Apivar® (Amitraz). Perizin® (Coumaphos) hatte diesen Status bis 2005, wurde aber nie in den Handel gebracht. Seit einigen Jahren verwenden viele Imker Asuntol®50 (Coumaphos). Dieses Tierarzneimittel hat keine Zulassung für die Anwendung in Bienenvölkern. Diese Bestandsaufnahme soll eine Übersicht über die Akarizid-Belastung von Handelswachs in Frankreich geben. In Zusammenarbeit mit der Bienenwachs verarbeitenden Industrie wurden auch Proben vor und nach dem Umarbeitungsprozess von Altwaben untersucht.
Bei zwei Völkergruppen mit je 5 Bienenvölkern wurde darüber hinaus die Rückstandsentwicklung von Amitraz und Coumaphos in Wachs und Honig nach der Anwendung von Apivar® (2 Streifen pro Volk, Anwendungsdauer 10 Wochen) bzw. Asuntol® (10 g einer Mischung Asuntol®50 / Puderzucker 15:500) verfolgt.
Für die geplanten Untersuchungen wurden spezifische HPLC- und GC-Methoden entwickelt. Coumaphos und Fluvalinat konnten in 89 % bzw. 70 % der Handelswachsproben (n = 47) mit Rückstandsgehalten von 0,27–5,81 $mg.kg^{-1}$ bzw. 0,13–3,62 $mg.kg^{-1}$ nachgewiesen werden.

Sämtliche Proben, die während der Umarbeitungsphase von Altwaben gezogen wurden, waren mit Coumaphos und Fluvalinat belastet.
Nach Apivar®–Behandlung wurden Amitraz-Rückstände im zentralen Bereich der beiden Waben gefunden, die 24 Stunden lang Kontakt zu den Streifen hatten. In der Folgezeit konnte der Wirkstoff in keiner anderen Probe des Bienenvolkes (Wachs der peripheren Waben und im Honig) nachgewiesen werden. Nach Asuntol®50-Behandlung nahmen die Coumaphos-Rückstände im Bienenvolk zu. Der Wirkstoff war im Honig mehr als 30 Tage und im Wabenwachs über 1 Jahr lang nachweisbar. Einen Tag nach der letzten Asuntol®50-Anwendung lag die durchschnittliche Coumaphosbelastung im Wachs bei 16,1 $mg.kg^{-1}$ und erreichte nach 26 Tage einen Wert von 6.9 $mg.kg^{-1}$. Die Halbwertszeit von Coumaphos lag im Honig bei 69 und im Wachs bei 115–346 Tagen.
Diese Ergebnisse bestätigen bzw. vervollständigen die Ergebnisse vorangegangener Studien. Die Verwendung von Coumaphos führt zu einer lang anhaltenden Kontamination des Bienenwachses. Die hohe Persistenz des Wirkstoffs führt im Rahmen der industriellen Umarbeitung von Altwaben zu kontaminierten Mittelwänden, die mitverantwortlich dafür sind, dass Coumaphos-Rückstände auch in Imkereien gefunden werden, in denen der Wirkstoff nicht zum Einsatz gekommen ist.
Da eine negative Wirkung dieser Rückstände auf die Gesundheit der Bienenvölker nicht ausgeschlossen werden kann, sollte auf eine Asuntol®50-Anwendung verzichtet werden.

Honig / Wachs / Akarizide / Rückstände / Kontamination

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