ORGANIC BEEKEEPING AND ACARICIDE RESIDUES IN BEESWAX. RESEARCH IN THE LAZIO REGION (CENTRAL ITALY)

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Summary

The problem of acaricide residues in beeswax from organic beekeeping was investigated, through the analysis of different wax matrix:

- *some samples of certified organic wax resulted not completely free from residues;*
- *cappings wax from conventional apiaries showed an average residue content of 0.7 mg/kg (maximum value 2.4);*
- *brood combs from conventional apiaries treated with perizin and asuntol, showed a much higher residue content, mostly between 1 and 10 mg/kg;*
- *cappings and brood combs from organic apiaries resulted mostly under 0.5 mg/kg;*
- *combs newly constructed by bees on foundations with different acaricide content showed on average the third part of the initial residue value. However, the residue content may increase if the foundation sheet is placed close to old contaminated combs.*

These results confirmed that the presence of residues in beeswax is a very critical issue for the organic beekeeping. To meet the conditions of the EC Regulation 1804/99, considerable caution and controls are necessary in replacing nest wax. In organic beeswax a maximum residue limit of 0.5 mg/kg seems to be acceptable.

Introduction

The research is part of a project launched by the Regional Administration of Lazio (Central Italy) in 2000-2001, in order to monitor organic farms in the area, and evaluate, on an experimental basis, the possible critical points of the EC Regulation 1804/99 in its first phase of application.

The EC Regulation prescribes for organic beekeeping the absence of treatments with conventional drugs against bee pathologies. In particular, conventional acaricide treatments against Varroa often result in a long-lasting contamination of beeswax, and for this reason one of the focal elements during the conversion period is the complete substitution of the nest wax with organic wax. If organic wax is not available, the use of wax from cappings is allowed.

One of the main points investigated in this study was the presence of drug residues in beeswax, through an experimental approach where the analytical study (GC/ECD) was connected with the beekeeping practice in an apiary under conversion.

Sample preparation and analytical method

Wax samples were analysed following a step procedure. Combs are pressed to eliminate honey, eventually present; combs and/or beeswax are melted and filtered in a thermostatic oven at 75 °C by means of a conic funnel with a 5-6 cm hole closed with filter paper; 0,2 g of beeswax are dissolved with hexane in a heater block at 60 °C and then centrifuged

three times at –10 °C at 5000 rpm for 15 minutes. The liquid extracts are purified on a 500 mg Florisil SPE column. Analysis is carried out on a GC/ECD system with a J&W DB-1 capillary column (Wallner, 1993).

Quantification limits for the residues in beeswax are 0.05 mg/kg for coumaphos and 0.01 mg/kg for fluvalinate.

Analysed matrix and results

1. Constitution of an organic apiary and reliability of “certified wax”

In 2000 the constitution of an experimental apiary complying with the provisions of the EC Regulation was initiated. As a first step, it was necessary to replace nest wax with organically produced wax or, according to the derogation, with cappings wax. Therefore, a sample of cappings wax from conventional apiaries and a sample of certified organic wax purchased on the market were sent to a laboratory, for residue analysis, in order to ascertain their suitability. A sample of wax from brood combs was sent too, to evaluate the initial contamination level of the hives. Since the analytical results showed a very high acaricide content, even in the “organic” wax, samples of the same three waxes were sent to other private and public laboratories, to verify the reliability of the analytical results.

Coumaphos and fluvalinate values found in the three samples by the different laboratories, our included, resulted quite different (Table 1) and, even if these results could in part be attributed to a possible not complete homogeneity of the samples, (that were not intended for a ring test), they make evident the need for a standardisation of the methods.

Anyway, the “certified organic wax” (which is expensive and not easy to find on the market) resulted not really free from residues and, on the other hand, cappings wax do not appear as a suitable alternative.

Table 1 – Analytical results of different laboratories on the same 3 wax samples (<dl: under detection limit. n.a.: not analysed).

Laboratory		1*	2	3	4*	5	6	7
Quantification limit declared by labs (mg/kg)		0.02	0.01	0.01	0.02	0.05	not given	not given
sample 1 (organic wax)	Coumaphos	<dl	0.88	0.22	0.27	0.25	0.49	0.15
	Fluvalinate	<dl	0.46	0.35	<dl	0.20	n.a.	n.a.
sample 2 (cappings wax)	Coumaphos	-	6.78	1.36	1.67	1.57	3.25	2.07
	Fluvalinate	-	0.11	<dl	<dl	<dl	n.a.	n.a.
sample 3 (brood comb wax)	Coumaphos	-	16.6	5.49	9.64	4.80	11.90	n.a.
	Fluvalinate	-	0.69	0.86	<dl	0.26	n.a.	n.a.

* Lab # 1 is the one who had released the certificate for the organic wax; # 4 is the same lab.

2. Evolution of acaricide residues in the hive

Several tests were performed to investigate the evolution of coumaphos residues in beeswax, from the foundation sheet to the comb newly constructed on it, and the effect of contiguous contaminated old combs on the residue content of the newly constructed comb.

Foundations with different coumaphos content, or empty frames without foundation, were inserted in hives to be constructed by bees. The coumaphos content of the newly constructed combs was measured and compared with the initial content of the foundation sheets. Two series of trials were carried out, inserting the new foundation sheets into empty hives, with an artificial swarm, and into conventional hives close to old combs with a coumaphos content of about 5 mg/kg. Results are reported in Table 2.

Table 2 - Evolution of coumaphos (CM) content in wax, from the foundation sheets to the combs newly constructed on the same sheets, and effect of contaminated combs close to newly constructed combs (mean and min-max values).

Type of foundation	Initial CM content in the foundation sheets (mg/kg)	Newly constructed combs (empty hives)		Newly constructed combs (CM in close combs about 5 mg/kg)	
		N. samples	CM content (mg/kg)	N. samples	CM content (mg/kg)
organic wax *	0.25	9	<0.05	7	0.33 (0.10-0.49)
organic wax *	0.09	3	<0.05	4	0.26 (<0.05-0.63)
no foundation	-	3	<0.05	5	0.50 (0.22-0.77)
cappings wax **	1.57	4	0.56 (0.20-1.10)	-	-

* Bought on the market ** From conventional apiaries

In absence of contaminated old combs, foundations with a low residue level, as well as empty frames, yielded new combs free of residues. Foundations with a medium residue content (1.57 mg/kg) produced new combs containing on average the third part of the residues initially present in the foundation (values are quite variable, from 1/5 to 2/3).

In presence of contaminated old combs, with a coumaphos content of about 5 mg/kg, the new combs constructed on foundations with a low residue level, showed a coumaphos content higher than the initial one; the coumaphos content resulted even higher in combs constructed without foundation sheet (0.22 to 0.77 mg/kg).

Another trial was carried out in the apiary under conversion, to ascertain the time necessary for obtaining a ‘clean’ wax (Table 3). In 2001, cappings wax from a conventional apiary, with a 1.57 mg/kg coumaphos content, was used to make the foundation sheets for the honey super combs. After the honey harvest, the "second generation" cappings wax were analysed and showed a coumaphos content reduced to about the third part (0.43 mg/kg). This wax was used again to make foundations for the super combs. The third

generation cappings wax, collected after the summer harvest of 2002 resulted free from residues.

Table 3 - Evolution of residue levels in the cappings wax during two years. (mean and min-max values).

Year	Coumaphos content in super foundation (mg/kg)	Coumaphos content in resulting cappings wax (mg/kg)
2001	1.57	0.43 (0.39-0.48)
2002	0.43	<0,05

3. Investigation in apiaries of the Lazio region

In order to have an overview of the contamination level of apiaries in the Lazio region, forty-eight beeswax samples were collected and analysed:

- 30 from conventional apiaries, of which 22 from cappings wax and 8 from brood combs of hives treated with perizin or asuntol;
- 18 from organic apiaries, of which 9 from cappings wax and 9 from brood combs.

Results are reported in Table 4 and Fig 1.

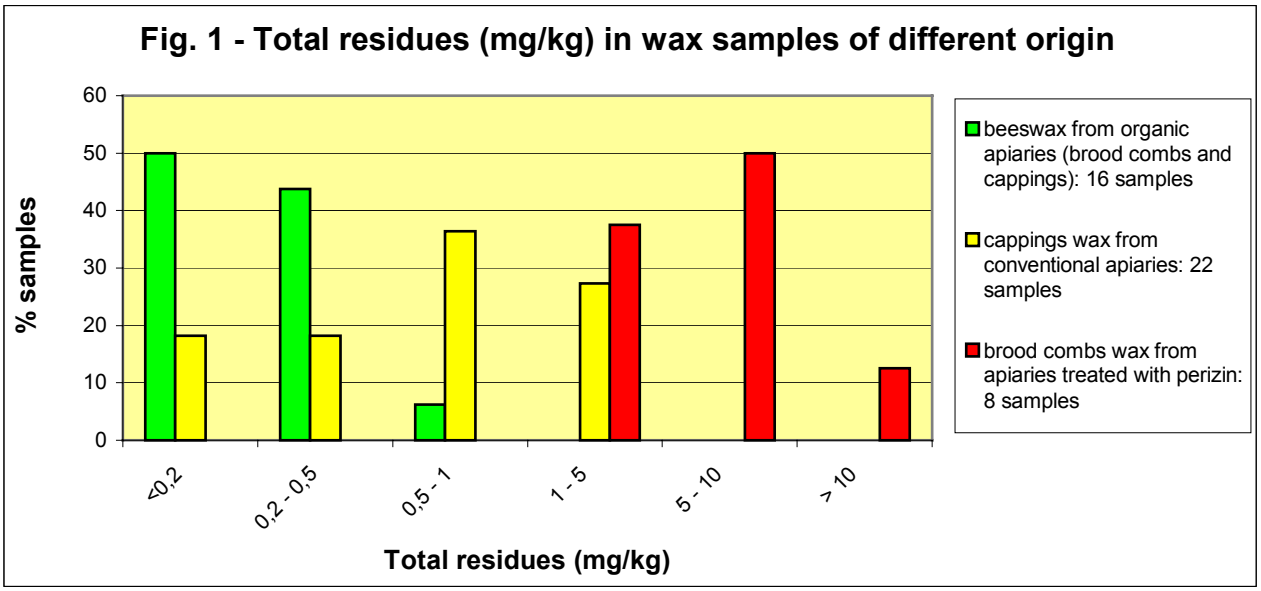
In conventional apiaries coumaphos residues were found in 18 cappings wax samples out of 22 and in all the 8 brood comb samples, often at very high levels. Fluvalinate residues were found in about the third part of the samples, as in nest that in cappings wax, confirming the long persistence of this product (Lodesani *et al*, 1992; Wallner, 1999) that has not been used in the region during the last years, due to the emerging resistance

Of the 18 samples taken from organic apiaries, two were excluded, showing a coumaphos/fluvalinate content considered not regular for organic beekeeping: 1.85/1.45 and 2.33/0.14 mg/kg respectively. In the remaining 16 samples, the maximum levels were 0.40 mg/kg for coumaphos and 0.37 mg/kg for fluvalinate, with no difference between cappings and nest wax.

EC Regulation has not fixed a limit for residues in organic beeswax, but usually in EU countries 0.5 mg/kg are considered acceptable, whereas in Italy a limit of 0.2 mg/kg is unofficially agreed by control organisms. In our tests, total residues exceeded 0.2 mg/kg in 50% of the 16 selected organic samples, whereas only one went beyond 0.5 mg/kg.

Table 4 - Residues in cappings and nest wax samples from organic and conventional apiaries: mean ± ds (min - max values).

	Coumaphos (mg/kg)	Fluvalinate (mg/kg)
Beeswax from conventional apiaries treated with perizin or asuntol: brood combs (8 samples)	6.89±4.95 (1.34 - 16.40)	0.14±0.16 (<0.01 - 0.36)
Beeswax from conventional apiaries: cappings (22 samples)	0.66±0.58 (<0.05 - 2.40)	0.10±0.19 (<0.01 - 0.75)
Beeswax from organic apiaries: brood combs and cappings (16 samples)	0.16±0.11 (<0.05 - 0.40)	0.08±0.10 (<0.01 - 0.37)



Conclusions

The results obtained give a fairly representative picture of the present situation of hive contamination in the Lazio region and, more generally, confirm that the presence of residues in beeswax is a very critical issue for the organic beekeeping. Some general considerations can be outlined.

1. Organic beeswax is not easy to find and not always it is really free from residues.
2. Due to an extensive situation of contamination of apiaries, conventional cappings wax is not a suitable alternative. Starting with a medium contamination level it seems that at least three cappings generations are necessary for obtaining wax free of residues (on average the decay rate from the foundation to the newly constructed comb is the third part of initial value).
3. Analytical methods for the determination of acaricide residues are not yet standardised and give a poor reproducibility, as confirmed by a recent ring trial performed by the International Honey Commission (Bogdanov and Bütikofer, 2002).
4. A maximum residue limit in beeswax should be fixed by the European Commission, to avoid differences in controls. A limit of 0.5 mg/kg in organic beeswax is usually considered acceptable in European countries, whereas in Italy only 0.2 mg/kg are allowed by the control organisms. In our analyses more than 40% of the organic samples have values between 0.2 and 0.5 mg/kg.
5. To meet the conditions for organic beekeeping, considerable caution and control appear to be necessary in replacing nest wax. The one year period prescribed by the EC regulation could be not sufficient for conversion, unless all combs are replaced at one time with new foundations of organic beeswax.

References

Bogdanov S., Bütikofer U., 2002 – Determination of acaricide residues in beeswax collaborative study. Report for the participants.

Council Regulation (EC) N. 1804/1999 of 19 July 1999 supplementing Regulation (EEC) N.2092/91 on organic production of agricultural products and indications referring there to on agricultural products and foodstuffs to include livestock production.

Lodesani M., Pellacani A., Bergomi S., Carpana E., Rabitti T., Lasagni P., 1992 - Residue determination for certain products used against varroa infestation in bees. Apidologie, 23: 257-272.

Wallner K., 1993 - Eine Method zur Bestimmung von varroaziden Ruckstanden im Bienenwachs. Apidologie, 24: 502-503.

Wallner K., 1999 – Varroacides and their residues in bee products. Apidologie 30: 235-248.