

# **Varroa jacobsoni** control by feeding honey bees with organic cupric salts

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## INTRODUCTION

Following the discovery by Popeskovic (1984) of differences in the copper composition of honeybees and their parasitic mite, *Varroa jacobsoni* O., it was shown that feeding bees with copper derivatives could exhibit a systemic toxicity to the parasite (Popeskovic and Bounias 1986). Previous experiments demonstrated that cupric sulphate controlled varroosis in field trials (Guiraud *et al.* 1989). This finding prompted us to try organic salts of copper (Dufour and Bounias 1991), which resulted not only in a much lower toxicity, but also in stimulating properties (Nectoux 1991). Such beneficial effects of a possibly toxic substance are related to hormetic effects or hormesis (Neafsey *et al.* 1988). This brings an advantage over most of the synthetic acaricides which generally exhibit a more or less marked toxicity to honeybees, even at low doses (Illarionov 1991; Cascino *et al.* 1989; M'Diaye and Bounias 1991, 1992; Sotnikov 1981). Here we report the results of our three-year study on the effects of cupric gluconate and cupric lactate given to bees in sucrose syrups, on the mite infestation levels of hives, on bee mortality, on feeding behaviour, and on copper residues in honey collected during the experiments. Since the efficacy of synthetic acaricides is considerably lower in the presence of brood (Romaniuk 1983), the influence of the proportion of brood combs among occupied ones also was studied.

## MATERIALS AND METHODS

**Hives and bees.** Dadant hives with 12 frames were used, together with a small number of Voirnot and Elite models. All experiments were performed in central France, near the town of Clermont-Ferrand, district of Puy de

Dome. All honeybees were *Apis mellifera mellifera* L. The entire experiment involved nearly 3,000 colonies managed by the Syndicat des apiculteurs du Puy-de-Dome. Observations were made on more than 1,500 colonies.

**Feeding techniques.** Comparative studies of the attractiveness of various cupric salts were made using special feeders. Tanks were subdivided into four compartments and placed in the upper part of the body of each hive. This allowed four different syrups to be simultaneously offered to the bees of one colony for comparative attractiveness. The occupied frames were positioned in the central part of the hive to concentrate bee activity in this area. Each test hive thus provided one replication for each of four different syrups in each of the four compartments. Attractiveness was evaluated as the number of days required for complete removal of the syrups. The control was sucrose syrup.

**Field treatments.** Sucrose solutions (1 kg per litre), with or without cupric components, were administered in spring (April to May) and in summer (July to August). Preliminary experiments had shown that a maximum ingestion rate was reached when the colonies were given the following successive volumes: during the first week, daily 0.25, 0.5, then 1.0 litre; during the second week, daily 1 litre; during the third week, an additional 2 litres; and finally 1 litre when needed for adjusting the total amount of copper metal to be ingested per hive. Concentrations of cupric derivatives were established.

**Toxicity to bees** — Adult bee mortality was observed over the duration of the experiment by periodically counting the dead bees both inside the hives (floating on the syrups or remaining at the bottom of the feeders) and outside on a 1 sq. metre plate situated on the ground at the hives' entrance. In all cases, data were

recorded in identical conditions for groups of hives situated in a single location.

**Efficacy against *Varroa*** — Specific studies were performed by periodic counts of dead mites at the bottom of the hives, on a special plate coated with glue and protected by a thin metallic screen which prevented their removal by the bees. Dose-related studies were conducted by weekly counts of dead parasites, after administration of the syrups, and then killing off the remaining population of living mites using a shock treatment either by amitraz or fluvalinate. Both of these acaricides gave similar results in comparable conditions, *i.e.* a 92±3% efficacy, determined after exhaustive counts in killed colonies (André 1988; Nectoux *et al.* 1988; Guiraud *et al.* 1989). The apparent efficacy (E) of feeding treatments was estimated for each colony over a 4±1 week or an 8 month period, *i.e.* short-term or long-term assays, by the ratio of the number of parasites killed during the feeding period (NF) to the total number obtained by adding this value to the number of remaining parasites following shock treatments (NC):  $E = NF / (NF + NC)$ .

An estimation of the relative efficacy percentage, with respect to controls receiving only sugar, was given by  $R\% = 100 \times (No - Ni) / No$ , where Ni is the number of parasites remaining after treatment at dose (i) of cupric compounds, and No is the corresponding number in control hives, representing the natural level of the parasite population under identical conditions. This ratio represents the proportion of falls likely due to the treatment. Note that total parasite falls (N) is hopefully higher in treated (NF) than in control (NC) hives, so that  $NF \gg NC$ , whereas  $No \gg Ni$ .

The number of brood combs (BrC) was recorded and compared to the total number of occupied frames (OcF),

including storage combs. The ratio  $StI=BrC/OcF$  was then used as an index of the strength of the colonies.

**Cupric derivatives.** Pure sucrose was provided by Sigma Chem., Inc. Cupric sulphate ( $CuSO_4 \cdot 5H_2O$ ) was prepared by La Cornubia (Bordeaux, France) according to the Macclesfield process. Cupric gluconate ( $C_{12}H_{22}O_{14}Cu$ , M.W.=453.8, 14% of Cu) and cupric lactate ( $C_6H_{10}O_6Cu$ , M.W.=241.7, 26% Cu) were synthesized by Benecchim S.A., Lessines, Belgium as blue water-soluble crystalline powders essentially free of heavy metals [less than 0.001% of lead and arsenic (U.S. Patent 1991)].

**Residues in honey-Copper residues** were determined in honey samples collected in the hives at the end of the treatments and at the normal period of harvesting. Copper concentrations were determined by atomic absorption photometry using a Perkin Elmer 420 model equipped with an HGA67 graphite oven.

**Statistical procedures** — Means were compared using either the Student's 't' test for normally distributed variables or the Mann-Whitney/Wilcoxon test for nonparametric statistics. Analyses of variance were performed with respect to controlled variables (or controlled factors), and the corresponding levels of significance against the residual variability were calculated for each involved factor using the 'F' distribution (Schwartz 1985). Statistical significances were assessed by taking into account the remaining numbers of replications. The final probabilities are valid for the corresponding numbers of freedom degrees.

## RESULTS

Attractiveness of syrups. The rates of consumption decreased in the following order: sucrose with cupric gluconate, sucrose alone, sucrose with lactate, and sucrose with sulphate (Table 1). Stronger colonies consumed syrup more rapidly, and the analysis of variance showed a

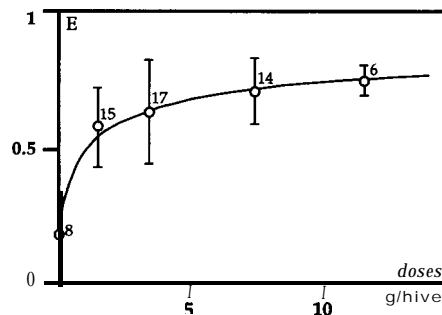


Figure 1. Global plot of dose-related efficacy (E) of cupric gluconate treatments (g/hive). Vertical bars represent the S.D. for independent results obtained from the number of individual colonies, given with regard to each point (total number of hives:

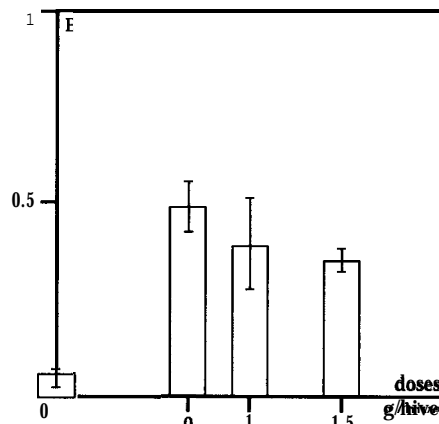


Figure 2. Dose-related efficacy of cupric lactate treatments (g/hive). Dose zero corresponds to controls with sucrose only. Means  $\pm$  S.D. (vertical bars) are given for  $n=3$  replications. The average number of mites per hive was  $104 \pm 23$  (total number of hives: 12).

highly significant influence for 'types of syrups' and 'strength index' ( $P < 0.001$ ).

**Mortality of bees and hives.** Data were recorded for various doses, up to a total 12.5g of cupric gluconate ingested per hive, at various periods of the year. Following analysis of variance, there was no effect of cupric gluconate concentration in sucrose syrups, whereas the period of the year proved sig-

nificant ( $P < 0.001$ ) (Table 2). For the 1,265 colonies distributed in 8 locations, the annual loss was  $4 \pm 1\%$ , the same as in non-infested apiaries (data not shown).

**Dose-related effects of cupric gluconate.** These data were obtained from apiaries in which various infestation levels were observed. Using the whole set of data, only doses proved to be a significant factor ( $P < 0.001$ ) (Table 3). Dose-mortality data therefore have been plotted regardless of infestation rates. This curve allows an apparently asymptotic limit [ $E_{max}=0.78$  and a 50% efficacy dose  $ED_{50}=0.65 \pm 0.26$  g per hive] to be calculated from the Hill equation describing the phenomenon (Bounias 1989, Figure 1).

The lack of influence of infestation levels, however, essentially was due to the effects of the higher dose (not used in practice) at the higher infestation level. These data excepted, the results exhibited a statistically comparable efficacy up to 408 mites per hive and from 1.5 to 7.5g of total copper gluconate ingested per hive; i.e. 0.2 to 1.0g copper metal ingested per hive.

**Dose-related effects of cupric lactate.** No dose-dependent differences were observed for the mortality of mites in experiments performed on a series of hives showing comparable infestation levels. From 0.7 to 1.5g per litre of cupric lactate, i.e. 0.18 to 0.39g of copper metal ingested per hive, the effects are significant regardless of concentrations (Figure 2).

**Kinetics of parasite mortality.** Short and long term experiments were conducted in highly infested zones. Following the first four weeks of treatment, the rate of parasite falls reached a constant for about 6 months (Figures 3 and 4). In a 1-year study, 5 hives were successively treated with 3g cupric gluconate per hive in April, 5g in

Dose (Cu/hive):	Controls 0.00g	Cupric additives		
		Gluconate 0.079	Lactate 0.13g	Sulphate 0.12g
Mean strength index (StI)				
0.40	3 (1)	3 (1)	5 (1)	6 (1)
0.42	2 (2)	1 (2)	3 (2)	4 (2)
0.45	2 (2)	2 (2)	3 (2)	4 (2)
average:	2.33M.58	2.00 $\pm$ 1.00	3.67k1.15	4.67 $\pm$ 1.15

Total observations:  $n=20$  in four hives  
 Analysis of variance: Influence of factor 'cupric salts':  $F=130$  ( $P < 0.001$ );  
 Influence of factor 'strength':  $F=111$  ( $P < 0.001$ )

Table 2  
Honey bee mortalities at various periods versus the amounts of cupric gluconate ingested per hive. Data represent the numbers of dead bees recorded in similar conditions over the period of treatments. The numbers of hives we in parentheses. One\* of organic salt contains 0.14g of pure copper metal. Doses actually applied are given  $\pm$  their S.D.

Doses g/hive	Dates			
	March	April	April/May	July/August
3.0	150.0 $\pm$ 19.8 (2)	145.5 $\pm$ 24.7 (2)	32.5 $\pm$ 10.5 (2)	12.5k2.5 (2)
1.5 $\pm$ 0.3	ND	118.3k32.6 (3)	33.3k5.8 (3)	11.0 $\pm$ 4.1 (4)
3.5M.2	129.7 $\pm$ 51.1 (3)	139.3f62.3 (3)	50.3 $\pm$ 15.6 (4)	22.2k7.1 (3)
7.0k0.4	152.0k73.4 (3)	188.0 $\pm$ 29.6 (3)	20.5f17.5 (4)	14.7k6.3 (3)
12.5 $\pm$ 1.0	203.7 $\pm$ 46.5 (3)	39.0 (1)	ND	ND

Total hives:  $n=48$ .  
 influence of factor date:  $F=54.9$  ( $P < 0.001$ ).  
 influence of factor dose:  $F=1.57$  (not significant).  
 ND: not determined

August, and 3g in March. High parasite mortalities were observed in all colonies (Table 4).

It should be emphasized that no massive falls of parasites were observed immediately after treatments, as opposed to the usual effects of synthetic acaricides.

**Influence of brood.** The variations of the efficacy ratio (E) versus strength indexes (StI) at constant doses were recorded during long term and short term studies. None of the variations observed within the range of our experimental conditions reached significance (Figure 5). The proportions of brood combs varied from the beginning to the end of treatments, and the calculated differences (d), representative of the hives' changes in strengths, were plotted versus administered doses of cupric gluconate. The maximum occurred at the smaller dose (1.5g cupric salt per litre of sucrose syrup) but, although the increase reached as much as 95%, the large variability of the data masked any significance (P=0.21) (Figure 6).

**Honey crop and copper residues.** The results were obtained from specimen experimental hives reserved for this purpose. Immediately after treatments, the concentrations of cupric gluconate in storage products (not yet properly honey) were proportional to given doses (Table 5). The regression calculation gave:  $r=0.997$ , with a slope  $b=5.0\pm 0.4\text{mg/kg}$  in honey per g given to hives, and an intercept:  $a=-0.18\text{mg/}$

kg with controls excepted. One month later, all concentrations were back to normal levels without any significant influence of concentrations within the range of practice. Following a three year campaign, the yield of honey was as high in treated hives and only slightly lower in controls situated at the same location.

## DISCUSSION & CONCLUSIONS

Cupric gluconate was the most attractive copper compounds we fed to bees which was not toxic to bees, even at high dosages. Mortality rates appeared to be related to seasonal factors, while mite control rapidly increased with cupric gluconate doses and varied with infestations levels. These points are consistent with the prominent role of season on the impact of the infestation (Kovac and Crailsheim 1988). It is noteworthy that the lower dose provided good efficacy indexes together with even lower mortality of bees than in controls. With cupric lactate, good control also was obtained at low doses, which may compensate for the lower attractiveness of this compound, although lactate does not seem to be superior to gluconate. Taking into account the large number of parasites present in other (private) apiaries situated in the surroundings of our own, at  $3,000\pm 2,000$  varroa per hive (Molaire 1990), the final relative efficacy of long term treatments with cupric organic salts ranges from

84.7% to 96.5%. The absence of influence of the proportion of brood combs suggests that feeding treatments

might affect the parasites in the overpopulated (i.e. closed) cells, or in proportion as they emerge from them. This is probably because of the longtime presence of cupric salts in the storage food, as attested by the sustained rate of parasite mortality after feeding has stopped, and by the determinations of copper residues in storage food. The presence of higher than normal levels of copper in control hives immediately after treatments suggests that some exchanges occurred between workers of different hives. It is interesting to note that the lower gluconate dose yielded the higher increase in the number of brood combs. This represents a strengthening of the corresponding colonies, which might correspond to the improvement effects observed in the lifespan of the bees (Nectoux 1990), and therefore fall into the category of hormetic effects. This contrasts with the toxicity of pyrethroid compounds, such as esfenvalerate, to honey bees (Mayer et al. 1990).

Taken together, these data show that feeding with copper-containing syrups provides a median and long-term inhibition of parasite development with no apparent damage to bees and no toxic residues in honey, where the natural levels of copper (about  $1\text{mg/kg}$ ) are not exceeded. Indeed, cupric treatments are long-term ones, and their effects cannot be compared to those of shock-treatments which use synthetic acaricides. However, the absence of toxic residues remaining in honey represents a substantial advantage, since traces of such pesticides as amitraz and fluvalinate are now found (Hemmerling, Augustinyak and Risto 1991; Sancho et al. 1991), thus lowering the quality of honey and raising a potential risk to consumers.

**Table 3**  
Influence of cupric gluconate dosage on the mortality of the parasite *Varroa jacobsoni* at various infestation levels of the hives. Doses are expressed in g of gluconate (that is 0.14g of copper metal per hive) ingested per hive over one month (1 mth) or one year (1 yr) periods. Means  $\pm$  S.D. are for the numbers of hives indicated between parentheses.

Doses	Infestation rates (average number of mites per hive)			
	3 $\pm$ 1 1 mth	19 $\pm$ 12 1 mth	408 $\pm$ 290 1 mth	1400 $\pm$ 780 1 yr
0.0	0.33 $\pm$ 0.24 (2)	0.21M.15 (2)	0.02M 01 (2)	0.14 $\pm$ 0.12 (2)
1.5 $\pm$ 0.3	0.47 $\pm$ 0.21 (3)	0.66 $\pm$ 0.41 (3)	0.44 $\pm$ 0.11c (4)	0.76 $\pm$ 0.03a (5)
3.5 $\pm$ 0.5	0.50 $\pm$ 0.50 (3)	0.67 $\pm$ 0.29 (4)	0.45 $\pm$ 0.11 (4)	0.91 $\pm$ 0.01a (6)
6.5 $\pm$ 1.1	0.72M 48 (3)	0.58M 28 (4)	0.66 $\pm$ 0.16b (4)	0.87 $\pm$ 0.13a (3)
11.5 $\pm$ 1.0	ND	0.75 $\pm$ 0.05c (3)	ND	ND

Total hives: n=...  
Significance levels in comparison with the corresponding controls (doses zero): (a) P<0.001. (b) P=0.003. (c) P=0.005. ND: not determined.

**Table 4**  
Long term effects (1 year) of treatments with a total of 11g cupric gluconate (1.5g copper metal)

treatment	number of mites killed	total number of mites	efficacy index	number of hives
controls	169 $\pm$ 50	1410 $\pm$ 421	0.12 $\pm$ 0.10	(3)
Copper	172.8 $\pm$ 9.0	227.0 $\pm$ 78.0	0.76 $\pm$ 0.03	(5)

relative efficacy R = 83.9%

Total hives: n=8

**Table 5**  
Honey yields (kg per hive in one year following 3 years of treatment) and copper residues in treated and control hives. Means  $\pm$  S.D. are given for (n) hives situated in identical conditions. Cupric gluconate concentrations are expressed in g per hive. Residues are given in mg of copper metal per kg of honey  $\pm$  S.D., calculated from independent samples.

Doses	Yields	Residues at the end of treatment	Residues 1 month after end of treatment
0.0	33.0 $\pm$ 7.8 (3)	5.2F1.7 (6)	0.67 $\pm$ 0.09 (4)
1.35	ND	ND	0.69 $\pm$ 0.10 (4)
3.2 $\pm$ 0.2	45.6 $\pm$ 30a (3)	18.1 $\pm$ 3.7 (12)	0.84 $\pm$ 0.34 (4)
5.0	44.6 $\pm$ 15.2 (3)	ND	ND
6.75	ND	31.0B.9 (12)	0.66 $\pm$ 0.02 (4)
13.5	ND	67.5 $\pm$ 20.6 (12)	ND

Total hives: n=67  
Significance level: (a) P=0.03.  
ND: not determined

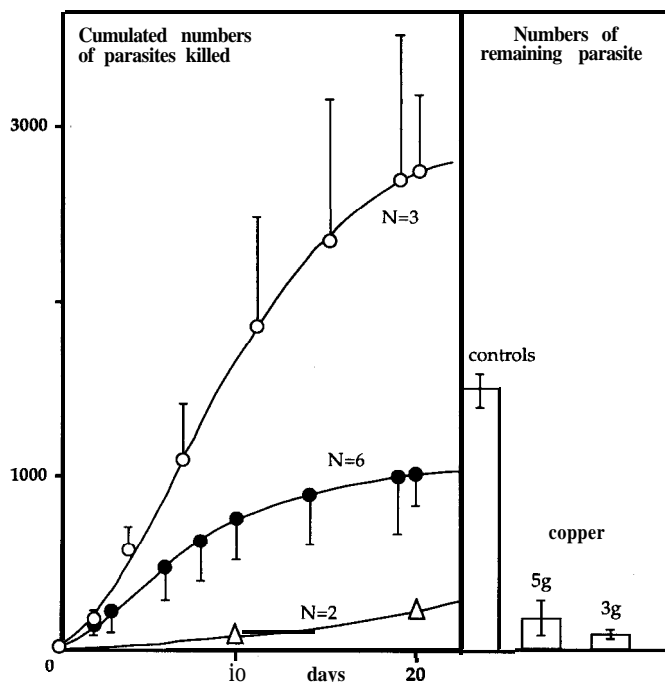


Figure 3. Kinetics of mortality of the parasites during treatments with copper gluconate. Short term studies. Means±S.D. are given for controls (D)(n=2) 5g per hive (O)(n=3), and 3g per hive (n=6). Histograms illustrate the compared remaining number of mites per hive at the end of treatments (total number of hives: 11).

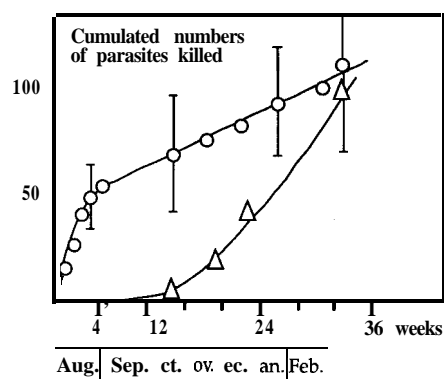


Figure 4. Long term studies with feeding 9.5g cupric gluconate in 5 litres syrup. Means±S.D. are given for n=6 hives. Controls (D). Copper (O) (total number of hives: 12).

The low toxicity of copper, which is essential to mammals (Abdel-Mageed and Oehme 1990; Syracuse Res. Corp. 1991), allows it to be incorporated in human food as a fungicidal preservative (Liang 1990). Moreover, copper gluconate has the advantage of meeting the specifications of the U.S. Food Chemical Codex (U.S. Pharmacopeial Convention 1981), which places its safety qualities far above other treatments with pesticides. Even feeding solutions at 0.028 to 0.28g of copper metal per litre are well below the doses which cause toxicity to pigs by ingestion (Abdel-Mageed and Oehme 1990). The purity of the cupric salts used in

our experiments (lead concentration lower than 6µg/g) was below the U.S.P. specifications published before 1991, 'less than 10µg/g' instead of 'less than 20µg/g' after October 1991 (United States Pharmacopeia 1981). The mortality of mites in colonies which were fed pure sucrose syrups might be related to the inhibitory role of sucrose on mitochondrial respiration, which would weaken the parasite's physiology and adversely affect its biological resistance (Nicholls and Lindberg 1972). Indeed, energy and water regulation by honey bees plays a role in the uptake of syrups (Skalicki *et al.* 1988). Treatment and feeding methods may have to be adapted to each country and season. ■

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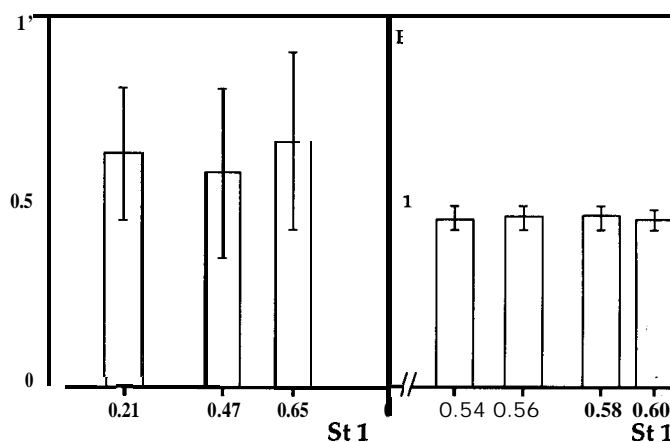


Figure 5. Relationships between the presence of brood and the action of copper. Variations of efficacy indexes (E) are plotted versus brood indexes (StI) in short term (left) and long term (right) studies. Respective doses of cupric gluconate are: 5.1±0.8 and 1.0±0.1g/hive. Means±S.D. are given for at least n=4 to 5 replications (total number of hives: 28).

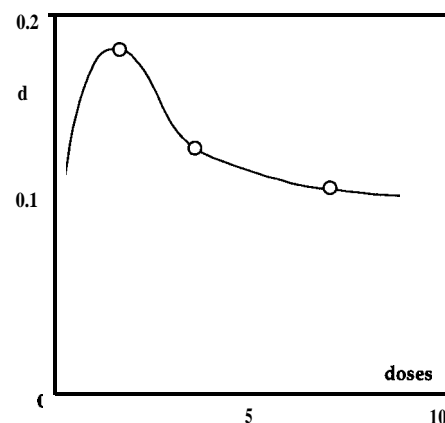


Figure 6. Stimulating effects of treatments. Relationship between the increase in strength indexes from the beginning to the end of the treatments (d) and doses of cupric gluconate (g/hive). Coefficients of variation are: CV=1.6. (total number of hives: 25).

#### ABSTRACT

For three years, about 1,500 hives were surveyed for varroa mites following feeding tests using cupric gluconate and cupric lactate in sucrose syrup. Three to five litres per hive of 0 to 2.8g of copper metal per litre of sucrose syrup were given in spring and/or summer. Bee and mite mortalities were recorded up to four years without significant toxicity being noted for honeybees. By contrast, up to 91% of the mites were killed in a dose-dependent manner by gluconate. Lactate was markedly less active than the gluconate, although it also prevented reinfestations. Cupric gluconate in sugar syrup was as attractive, if not moreso, than pure sucrose, and the copper concentrations in honey at harvest were unchanged. Efficacy of treatments was not brood dependent. Cupric organic salts therefore provide a safe way for preventing the infestation of hives and the development of the mite over long periods.

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**KEY WORDS**

Honey bees, varroa control, cupric gluconate, cupric lactate, feeding behaviour, toxicity, copper residues in honey.

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