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Fluvalinate Residues in Honey and Beeswax after Different Colony Treatments

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Fluvalinate (D-isomer) a synthetic pyrethroid, is a potent, broad range, foliar applied insecticide and acaricide, effective against major arthropod pests of various crops. It was first presented as a promising agent against Varroa mite as spray solution in water (Borneck, 1986) and as impregnated comb foundations (Koeniger and Chmielewski, 1986). Since then, this acaricide has been extensively used worldwide by beekeepers in honeybees to control the parasitic mite *Varroa destructor* and prevent varroatosis. The registered fluvalinate formulation for apiculture is Apistan (Sandoz), which has the form of plastic strips (800 mg a.i. per strip), out of which a small amount of fluvalinate is slowly released and is actively dispersed into the beehive with the bees' legs by contact. The recommended application of fluvalinate in apiculture is the placement of two Apistan strips into the colony for a period of 4-8 weeks once or twice per year. During the treatment only a small portion 5-10% of the total acaricide diffuses out of the plastic strip (Bogdanov et al., 1998). However, non-authorized applications of an aqua flow formulation of fluvalinate are often made, as beekeepers apply fluvalinate either by spraying aqueous suspension of Mavrik, usually at a rate of 1.2-7.2 mg a.i./colony twice per year or by using home made wooden inserts dipped in aqueous suspension of agricultural formulations. The technique used to apply acaricides in beehives, the duration of the treatment, the season and the activity of the bees, could affect the residues remained in bee products. Repeated treatments or extended exposure times of fluvalinate formulations result in increased residues of this acaricide, mainly in beeswax (Bogdanov et al., 1998; Wallner, 1999; Menkissoglu-Spiroudi et al., 2001).

Several publications report on fluvalinate residues in hive products in various countries (Lubinevski et al., 1988; Taccheo et al., 1989; Balayannis and Santas, 1992; Lodesani et al., 1992; Bogdanov et al., 1998; Wallner, 1999; Tsigouri et al., 2000), but very few report comparative results on residues remained after treatment with different techniques or formulations. Application of fluvalinate according to the recommendation does not seem to contaminate the honey seriously, but the use of agricultural formulations results in variable residue concentrations (Lubinevski et al., 1988; Taccheo et al., 1989; Balayannis and Santas, 1992; Lodesani et al., 1992).

In the present study the effect of (a) different treatment procedures and (b) the season, on fluvalinate residues in honey and wax was investigated. Three different methods (formulations, techniques and doses) of fluvalinate application were used in

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field trials, representing the current preference of the Greek beekeepers: Apistan strips, wooden inserts impregnated with a suspension formulation (Mavrik 24 SC) and spraying with same formulation. Our objective was to evaluate the impact of each method, in relation also to the season, on the presence and the concentration levels of this acaricide's residues in honey and wax.

MATERIALS AND METHODS

Apistan and Mavrik (Sandoz) were purchased from the retailed market. Fluvalinate certified reference standard was purchased from Dr. Ehrenstorfer GmbH (Germany). All solvents used for sample analysis were of residue analysis grade.

Two field trials were conducted; one in spring and another in autumn. Each trial involved 10 bee colonies (all in Langstroth hives with 10 frames each). One of the colonies was served as control, while the others, in triplicates, received one of the following treatments: (I) application of Apistan strips, corresponding to the recommended use of the acaricide in apiculture. Two strips were inserted in each hive between the 3rd and the 4th and between the 7th and the 8th comb. (II) application of plywood inserts impregnated with Mavrik 24 SC suspension. Ten plywood inserts (16.5 x 2 x 0.8 cm) were immersed in 250 ml Mavrik suspension containing 480 mg fluvalinate/L. The inserts were left to impregnate for 24 hours. One insert was used for each colony and it was placed between the 7th and the 8th comb. (III) spraying with Mavrik 24 SC suspension at a concentration of 36 mg a.i./L. The volume used per colony was 200 ml resulting in 7.2 mg fluvalinate per colony. Strips and wooden inserts remained inside the hives for the whole duration of the trials. The spring (23-3-1998) and the autumn (20-10-1998) trial lasted 5 and 4 months, respectively. Before the autumn trial, 3 empty brood combs were placed in positions 1, 4 and 8 in every colony involved.

Honey samples were collected before the treatments from all 20 colonies. Brood comb samples were taken from all 30 empty brood combs used. Samples were also collected at time intervals following the acaricide application as shown in Figures 1-3. During the spring trial only honey samples were collected and analyzed, while during the autumn trial fluvalinate residues were also determined in brood comb wax. For sampling of honey about 10 cm^2 of comb, which contained honey, was carefully removed, placed in a plastic bag and transported to the lab under refrigeration. In the lab the piece of comb was uncapped with a knife, placed in cheesecloth and put in a funnel. Honey drained into a glass vial. Whenever honey was crystallized the apparatus was placed in a 50 °C incubator. Sampling of brood combs involved the combs placed in the hives before the trial. About ¼ of the comb was removed, placed in a plastic bag and transported to the lab under refrigeration. The first three samples were taken from brood combs in position 1, the following two were taken from brood combs in position 4 and the last ones from brood combs in position 4 or 8, depending on colony condition. Samples were wrapped in cheesecloth and one by one boiled in water for 30-35 min. Melted wax was poured into a suitable container to solidify.

The gas chromatographic methods used for the quantitative determination of

fluvalinate residues in honey and wax have been previously described (Tsigouri et al., 2000; 2001). The mean recoveries of the methods were 90.25 ± 0.85 and 77.50 ± 1.57 %, while the limits of determination were 1 and 100 μg/kg for honey and wax, respectively.

The Statistical Package for Social Sciences SPSS 8.1 was used. Normality of distributions was checked by Kolmogorov-Smirnov and Shapiro-Wilk tests, and homogeneity of variances by Levene's test. To test the hypothesis that means were equal, analysis of variance (ANOVA), as well as multiple comparisons (Scheffe or Dunnett, and Games-Howell tests) were performed, depending on the case.

RESULTS AND DISCUSSION

Fluvalinate residues in honey samples obtained after different application techniques from the two field experiments conducted in spring and autumn are shown in Figure 1 and 2 respectively. Fluvalinate residue determinations, made in honey samples before the treatments, revealed, that the colonies used had been previously exposed to fluvalinate. Concentrations measured in samples from the control hive were constant throughout the trials (± 0.5 μg/kg). The maximum fluvalinate residue concentrations determined in honey during the treatments with Apistan strips and wooden inserts were 6 and 6.8 μg/kg, respectively. After spraying with Mavrik, the maximun fluvalinate concentrations appeared the 1st day after treatment reaching 39.4 μg/kg in one colony. The 1st month the concentrations were significantly lower and remained almost constant for the whole duration of the field trials.

Statistical analysis of the results obtained after the spring trial showed that data distributions within each treatment were not normal, and data variances were not homogeneous. However, data transformation [concentration**(-0.5)] proved that transformed data followed normal distributions and had homogeneous variances. Application of Scheffe test (Table 1) showed that during the spring trial no statistically significant difference between the 3 treatments was noted. Results obtained from the trial conducted during autumn showed that data variances for each treatment were not homogeneous. For this reason multiple comparisons by applying Dunnett and Games-Howell tests were performed. As shown in Table 2, the amounts of fluvalinate residues determined in honey from hives sprayed with Mavrik were higher and statistically significantly different (P=0.024) than those found in honey from hives that received the Apistan application. The difference between spraying and applying plywood inserts was boundary (P=0.059), while no difference was found between treatment with Apistan and plywood inserts.

The results obtained from the trial conducted during autumn showed that spraying the colonies with Mavrik suspension loads honey with residues more than the Apistan strips treatment. Application of plywood inserts impregnated with Mavrik suspension seemed to cause an intermediate contamination. The results of the trial during spring did not reveal any difference between the methods of application regarding honey contamination with residues. This is probably due to increased nectar flow during the warm months and subsequent dilution of the old honey with the newly produced. It should be emphasized, however, that, in both trials, a high

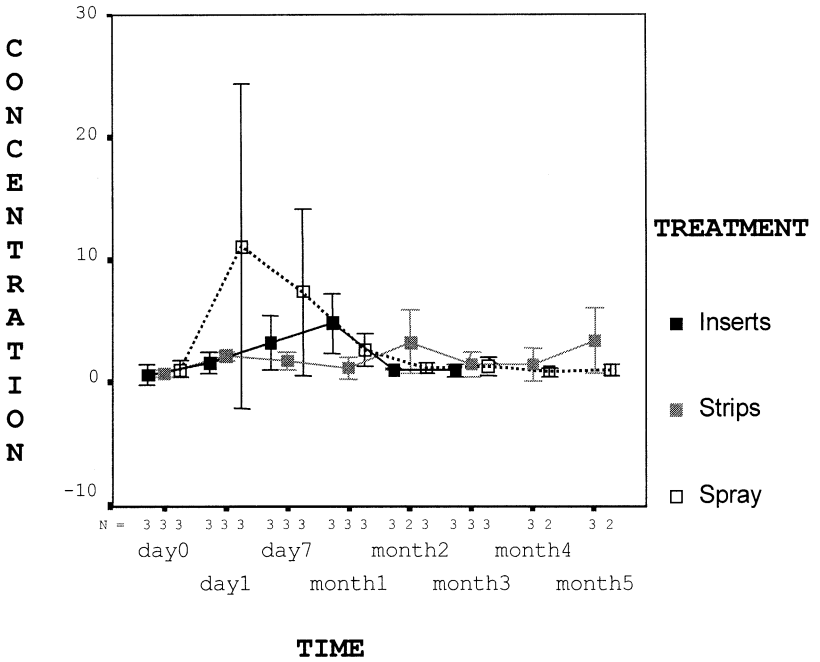

Figure 1. Fluvalinate residues in honey after different treatments in spring (means±2s.e.).

variation was found between colonies that had received the same treatment. Similar variability has been reported in the literature (Taccheo et al., 1989; Lodesani et al., 1992) and can be attributed to differences in bee activity. As fluvalinate is distributed throughout the hive by the bees' legs, factors increasing bee activity result in higher fluvalinate residue concentration in bee products.

Table 1. Fluvalinate residues in honey and multiple comparisons (Scheffe test) between the different treatments (spring trial).

Dependent Variable: Concentration					
(I) Method	**(J) Method**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confid. Interval**
woo	str	-3.79E-03	.073	.999	-.188 - .181
	spr	6.09E-02	.074	.711	-.125 - .247
Str	woo	3.79E-03	.073	.999	-.181 - .188
	spr	6.47E-02	.068	.638	-.107 - .237
Spr	woo	-6.0967	.074	.711	-.247 - .125
	str	-6.4758	.068	.638	-.237 - .107

woo: plywood inserts, str: Apistan, spr: spray

The maximum concentration of fluvalinate residues in honey, during Apistan treatment, did not exceed 6.1 μg/kg indicating that the use of Apistan strips seem to cause low contamination of honey. Althought low residues (Borneck and Merle,

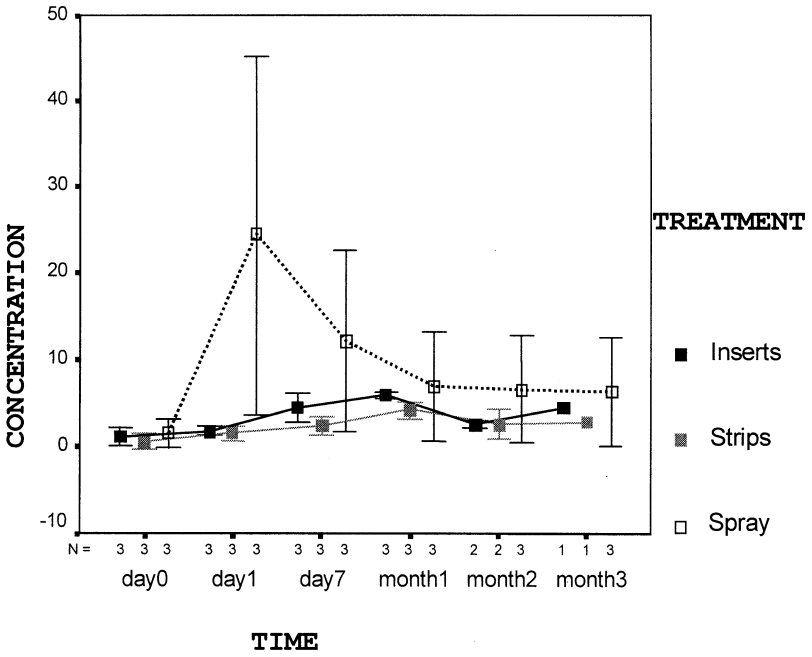

Figure 2. Fluvalinate residues in honey after different treatments in autumn (means±2 s.e.).

1989; 1990; Lodesani et al., 1992) or residue free honey (Taccheo et al., 1989) have been reported under various application conditions of Apistan strips, higher concentrations have also been reported (Taccheo et al., 1989; Lodesani et al., 1992). In all cases the duration of strips residence inside the hive affect the levels of residues. Varroa control with application of plywood inserts impregnated with Mavrik, has been first quoted by Lubinevski et al. (1988). They reported that honey samples from combs, which were in contact with the inserts contained fluvalinate at 57 μg/kg on the average, while the maximum value measured was 190 μg/kg. Since then, although the use of various carriers impregnated with Mavrik has been quite popular between the beekeepers worldwide, no other report has appeared regarding the residue levels in bee products. Under the conditions used during the field trials of this study, honey contamination was lower than the one reported by Lubinevski et al. (1988). Applying fluvalinate by spraying with Mavrik, the maximum residue concentration was measured the first day after treatment. Fluvalinate residues gradually reducing dropped to 1-2 μg/kg and 2.8-12.5 μg/kg at the end of the spring and autumn trial, respectively. The relatively high values that were measured one day after treatment are probably due to droplets of the Mavrik suspension that had fallen on uncapped honey. Balayannis and Santas (1992) reported higher residue levels (145.8 μg/kg) in spite of the fact that less amount of active ingredient/hive was used. Generally, the use of the agricultural formulation could result in variable residue levels depending mainly on the amount of the acaricide applied per colony.

The residues of fluvalinate in brood comb wax after the different treatments of the

autumn experiment are shown in Figure 3. Brood comb wax samples were obtained by melting in boiling water one quarter of each comb. In a previously conducted recycling experiment, it was established that this procedure does not lead to fluvalinate reduction, while it provides representative samples (Tsigouri, 2000). Measurements of fluvalinate concentrations in brood comb samples before the treatments showed that they all had been previously treated with the pyrethroid and were contaminated with residues at levels ranging from 0.2 to 32.8 mg/kg. Our results were expressed as the differences between the concentration at specified times and the corresponding initial concentration. Concentrations measured in samples from the control hive did not show high variation throughout the trial (+0.06 to + 0.27 mg/kg). Application of Apistan strips or plywood inserts caused a constantly increasing fortification of brood comb wax ranging from 0.18 to 3.86 mg/kg. After spraying with Mavrik, the maximun concentrations appeared the 1st day after treatment reaching 43.75 mg/kg. One week after spraying the concentrations of the acaricide were at the lowest levels and then they were slowly increasing. Four months after spraying the increase in wax concentration ranged from 0.79 to 12.69 mg/kg. Statistical analysis of the results showed that data distributions within each treatment were not normal, and data variances were not homogeneous. Transformed data [concentration difference**(-1)] followed normal distributions and had homogeneous variances. Application of Scheffe test (Table 3) showed that spraying with Mavrik resulted in significantly higher residue concentrations in wax than the application of Apistan strips (P=0.007), while no statistically significant difference was found between spraying and application of wooden inserts. The difference between treatment with Apistan and plywood inserts was boundary (P=0.082).

Table 2. Fluvalinate residues in honey and multiple comparisons (Dunnett and Games-Howell tests) between the different treatments (autumn trial).

Dependent Variable: Concentration

Tests	(I) Method	(J) Method	Mean Differ. (I-J)	Std. Error	Sig.	95% Confid. Interval
Dunnett T3	woo	str	1.081	2.108	.272	-.547 - 2.709
		spr	-6.341	2.019	.068	-13.080 - .396
	str	woo	-1.081	2.108	.272	-2.709 - .547
		spr	-7.423*	2.019	.027	-14.097 – (-.749)
	spr	woo	6.341	2.019	.068	-.396 – 13.080
		str	7.423*	2.019	.027	.749 – 14.097
Games-Howell	woo	str	1.081	2.108	.229	-.510 – 2.673
		spr	-6.341	2.019	.059	-12.895 - .212
	str	woo	-1.081	2.108	.229	-2.673 - .511
		spr	-7.423*	2.019	.024	-13.935 – (-.911)
	spr	woo	6.341	2.019	.059	-.211 – 12.895
		str	7.423*	2.019	.024	.911 – 13.935

woo:plywood inserts, str:Apistan, spr: spray,*:The mean difference is significant at the 0.05 level.

The results of the autumn trial showed that treatment of colonies by spraying with Mavrik suspension loads wax with higher amounts of fluvalinate residues than the use of Apistan strips, while application of plywood carriers impregnated with Mavrik suspension causes an intermediate fortification.

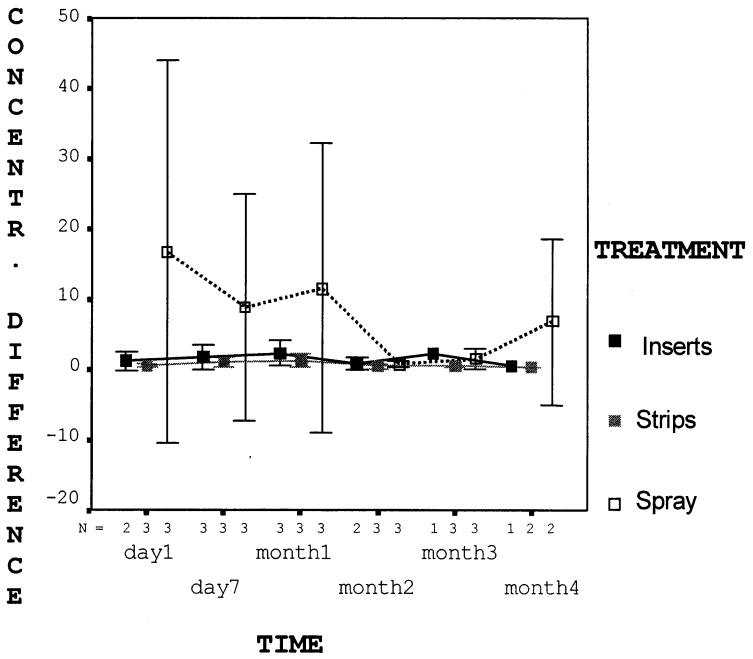

Figure 3. Fluvalinate residues in brood comb wax after different treatments in autumn (means ± 2 s.e.).

Table 3. Fluvalinate residues in brood comb wax and multiple comparisons (Scheffe test) between the different treatments (autumn trial).

Dependent Variable: Concentration					
(I) Method	**(J) Method**	**Mean Differ. (I-J)**	**Std. Error**	**Sig.**	**95% Confid. Interval**
woo	str	-1.271	.543	.082	-2.673 - .131
	spr	.441	.543	.721	-.961 –1.844
str	woo	1.271	.543	.082	-.131 – 2.673
	spr	1.712*	.494	.007	.436 – 2.988
spr	woo	-.441	.543	.721	-1.844 - .961
	str	-1.712*	.494	.007	-2.988 – (-.436)

woo:plywood inserts, str:Apistan, spr: spray,*:The mean difference is significant at the 0.05 level.

In the literature there have been other references regarding the detection of fluvalinate residues in wax due to application of Apistan strips (Borneck and Merle, 1989; 1990; Taccheo et al., 1989; Lodesani et al., 1992; Bogdanov et al., 1998), but there are no data referring to other application techniques. These results are contradictable and obviously depend either on the specific conditions of each experiment, or on the sensitivity of the analytical method used for fluvalinate determination. So, Borneck and Merle (1989, 1990) reported concentrations ranging from 0.2 to 0.8 mg/kg, Bogdanov et al. (1998) found mean values of 1.8 and 7.3 mg/kg, 1 and 5 months after application respectively, while Taccheo et al. (1989) did not detect residues 6 ½ and 8 ½ months after treatment. Also, a high variation was

reported between colonies that had received the same treatment, which can be attributed not only to differences in bee activity but to comb sampling as well.

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