EXPERIMENTS WITH APISTAN IN 1988

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A Review on the Previous Papers

Within the measures taken in controlling Varroa disease, several preliminary studies were made at the Institute*, in the period of 1985—1986, in order to establish the possible influence of Fluvalinat pyrethroid on adult honeybees and larvae. On this subject, several reports were delivered (comm. R. BORNECK at the Trisymposium from PHOENIX, Arizona, in December 1985, report by R. BORNECK to the Experts' meeting of EEC in October 17th 1986, report at the Symposium of Zagreb in November 1986, R. BORNECK and M. METAYER, home report ITAPI-ZOECON, R. BORNECK and B. MERLE, 1986).

A test was carried out by the end of 1986 and the beginning of 1987, in cooperation with a large number of beekeepers from all over Europe, on aprox. 10,000 honeybee colonies, and it confirmed for ITAPI the nontoxicity of the molecule when applied in quite small dosage on honeybees and its effect on *Varroa.* The reason why we didn't publish the results of the tests sent by beekeepers, is that these were disconnectedly presented. However, we have analysed them and would very much like to extend our thanks to all those, very many, who have sent them.

This wide cooperation has allowed us to continue other experiments. A significant mention is that none of our correspondents did notice, within field experiments, any abnormal reaction of the honeybee colonies after the treatment.

The experiments were continued in 1987 so as to test the efficiency of APISTAN acaricid on *Varroa jacobsoni* in infested hives, during winter. The studies also viewed an optimum placing of APISTAN strips inside the hives and compared the efficiency of formula depending on the percent of active matter — 10.20 and 30% (R. BORNECK and B. MERLE 1987 at the International Congress of Apiculture in Warsaw).

1. Introduction

The experiments in this report were carried out from June 6th to August 11th, 1988, after a well-ela-

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borated protocol written by Dr. Ducos de Lahitte from the Veterinary School of Toulouse. The studies he conducted were identical and were carried out in a site (Toulouse) which is different from the ecoclimatical point of view and honeybees *(A. mellifica ligustica)* different from ours *(A. mellifica mellifica),* in Langstroth hives instead of Dadant hives used within the experiments from Montbarrey. The experiments were required by the ZOECON company in order to make a file of the A.M.M. The experimental protocol was being studied by the biologist from the M. SHEATA-SHEATA company, with headquarters in Dallas, USA.

1.1. Objectives

The experiments had in view:

— to verify the acaricidal efficiency on *Varroa jacobsoni* mite using two strips of APISTAN of 8 grams, each one containing 10% active matter when introduced inside infested hives during summer and in the presence of brood, for 6 weeks.

— to verify the quality of the therapeutical treatment on the long run, to be undamageful to the honeybees.

— to study the eventual changes of behaviour of the honeybees during and after treatment as regards development, yield and evolution of the honeybee colony.

— to study the quantity of residues of active matter which may be found in honey and wax, in different stages of the treatment.

2. Material and Methods

2.1. Material

Those 40 hives used are Dadant type with 10 frames, provided with special devices which allow for dead mites collecting, those that have fallen off honeybees, on greased sheets of paper, easily to be taken out from the device for laboratory counting means.

2.1.1. Apistan strips are made of P.V.C. containing 10% active matter (Fluvalinat). Each strip measures 25 cm length and 3 cm width and 0.2 cm thickness. On average it weights 8 gr and contains 0.8 active matter. Two strips/colony were used.

2.1.2. The biologic material was made up of 40 honeybee colonies of *Apis mellifica mellifica.* Mean infestation rate was fluctuating but never smaller than two *Varroa* mites to 200 honeybees (visual counting after washing-immersing-stirring, filter through two superposed screens of various diameters).

2.2. Method

2.2.1. Experimental groups

The 40 experimental hives were numbered and divided in 4 groups of 10 hives each. They were evaluated in relation to the population level, brood, infestation. Afterwards, they were placed on the same bee yard to ensure identical ecoclimatic conditions to the experimental group.

Hives' allocation to various groups was done at random.

GROUP 1 (hives no. 3,5,6,16,21,23,26,29, 31,35)

These hives received two strips of Apistan between frames 3, 4, 7 and 8. The strips remained in the hive from day JO-6 June 1988 to day J+42 (July 18th 1988). On this date the colonies were destroyed and all *Varroa* mites remaining on honeybees and brood were counted.

GROUP 2 (hives no. 1, 4, 12, 17, 20, 25, 28, 33, 34, 37)

The hives from the second group received the same treatment with Apistan, however, starting from the 42nd day these received a treatment with Perizin (Bayer), two applications at 7 days period, according to the manner of usage of this drug.

GROUP 3 (hives no. 7, 8, 10, 18, 22, 27, 30, 36, 38, 40)

The 10 hives of this group represented the "treated control group". They received a treatment with Sherring antivarroa (Amitraz) in the day JO+7, J+14, J+21, according to the usage instructions of the company (aerosol for 1 minute and 30 seconds). The colonies were also treated (same like the hives of group 2) twice with Perizin in the days J+42 and J+49.

GROUP 4 (hives no. 2, 9, 11, 13, 14, 15, 19, 24, 32, 39)

The group received a treatment with Apistan just like the first three groups until day J+70 when the hives were put under strict observance to notice any eventual changes in the behaviour of the colonies, following treatment.

2.2.2. Special Dispositions

2.2.2-1. All hives are provided with a device which allows an easy removal through the back of the hives of the greased papers on which dead *Varroa* mites were sticked. There is a lattice work that impedes the access of the honeybees to these papers. These are taken out from the hives, folded, numbered and then transferred to the laboratory for the visual counting of the mites.

2.2.2-2. Counting the dead honeybees:

A white plastic 1 m sq. lattice work is placed in front of each hive to collect all dead honeybees and count them.

2.2.2-3. Closing queen honeybees in the cages:

In order to reduce the brood surface when destroying honeybee colonies from the first group and to facilitate the counting of *Varroa* mites in a smaller number of cells, the queens from the first, second and third group were closed in cages in the day J+28 and left in the colonies. The queens from groups 2 and 3 were released in the day J+45.

2.2.2-4. The statistic calendar of honeybees' and *Varroa* mites' mortality. Counting was made at two days interval from day J−3 to JO; every day from J+1 to J+8; every two days from J+8 to J+18; every four days from day J+18 to day J+42.

2.2.3. Wax and honey drawing and study on the residues

Honey (50) and wax (50) drawings were made at random in five hives from each of the 1, 3, and 4

groups in the days J−1, J+28, J+42 and J+70 in group 4. 3/5 of these samples were the object of an analysis.

3. Results and Discussions

3.1. The mortality of *Varroa* mites in various experimental groups was presented in complete tables.

Group 1

Table 1

Treatment with Apistan 2 Strips/Hive, for 6 Weeks
Hives' Destruction on Day 43rd

No. colonies	No. dead *Varroa* between J+1 and J+42	Destroyed colonies		Total no. of dead *Varroa*	Efficiency %
		No *Varroa* honeybees	No. *Varroa* brood		
3	792	1	0	793	99.87
5	2071	9	2	2082	99.47
6	329	6	1	336	97.91
16	3054	10	2	3066	99.61
21	1536	4	0	1540	99.74
23	1103	4	0	1107	99.64
26	2620	4	0	2624	99.85
29	4492	4	0	4496	99.81
31	442	1	2	445	99.33
35	1902	1	0	1903	99.95
					99.72=M

However, we publish only those tables (1, 2, 3, 4) which give overall results and the efficiency rate by the end of the treatment.

On the contrary, diagrams 1, 2, 3 and 4 show the mortality for each counting during experiment.

3.1.1. Mortality in group 1

The efficiency rate was calculated according to the traditional formula:

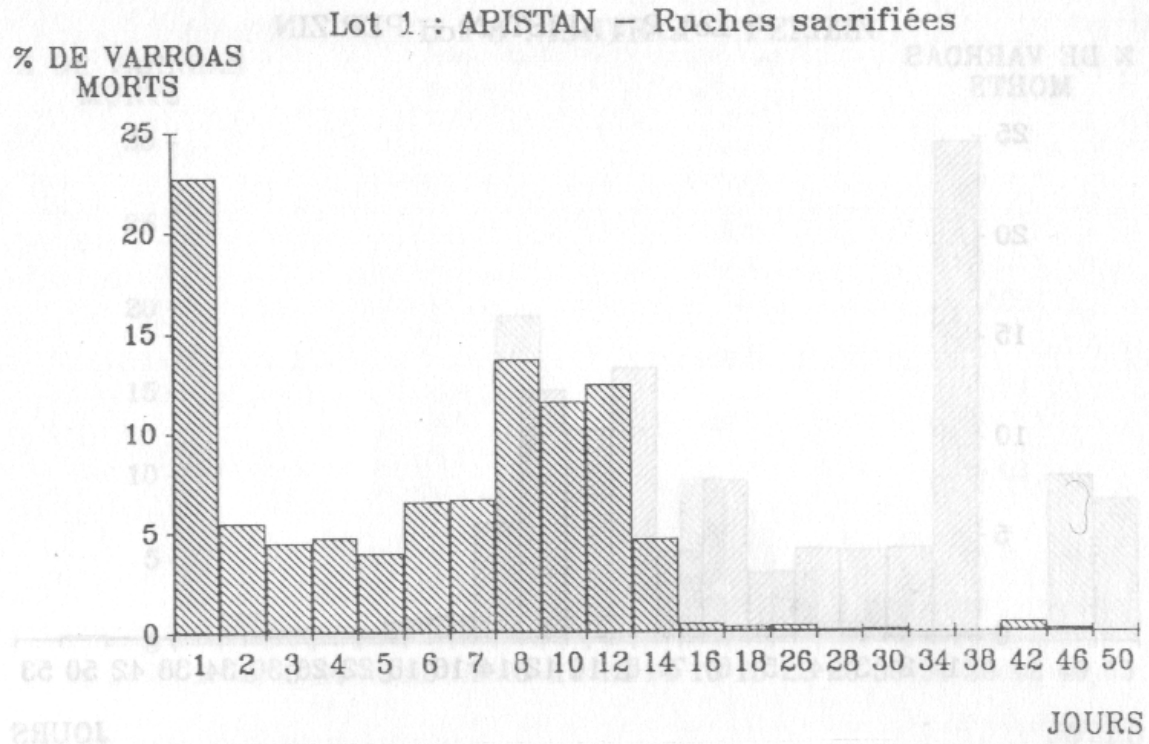
$$\text{Rate of efficiency} = \frac{\text{No. of dead Varroa mites from JO to J+42}}{\text{No. of dead Varroa mites from JO to J+42 + No. of dead Varroa after killing}}$$

7 *Varroa* mites were found after destroying the 10 colonies in 25,538 cells.

On those 134,398 dead adult honeybees were found 44 *Varroa* mites. Thus, efficiency of Apistan treatment was of 99.72% for this group. Undoubtedly, this group is the most interesting one since the possibility of a reinfestation is low, as the colonies are killed several hours after the removal of the strips (the percent mentioned to the end of the table does not correspond to an arithmetic mean).

3.1.2. Mortality in group 2

Like the first one, this group was also treated with Apistan. On the 43 rd day, after the first treatment with Perizin a mortality of 75 mites was registered; after the second treatment in the 53rd day, there was a



Group 2

Table 2

Treatment with Apistan 2 Strips/Hive, for 6 Weeks
Control Treatment with Perizin A, J+43 and J+50

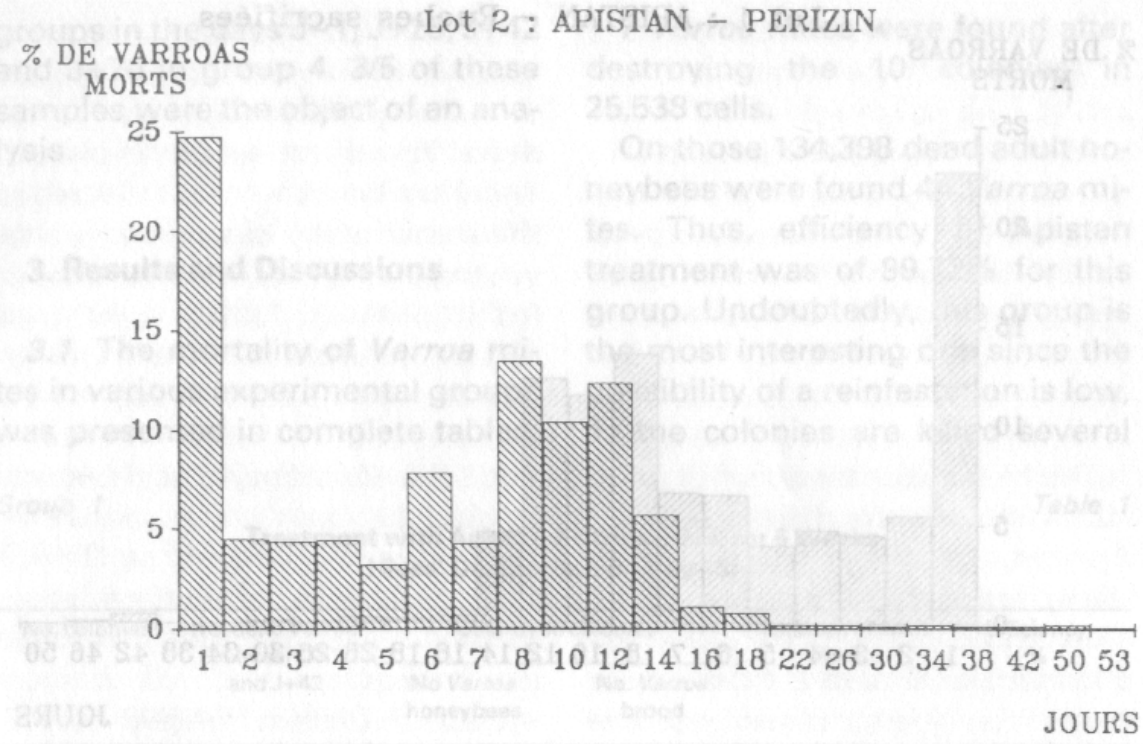
No. colonies	No. of dead *Varroa* between J+1 and J+42	Control treatment with Perizin No. dead *Varroa*		Total no. of dead *Varroa*	Efficiency %
		Application 1	Application 2		
1	4780	3	4	4787	99.85
4	2346	18	13	2377	98.70
12	2708	17	7	2720	99.12
17	1018	0	0	1018	100.00
20	1651	4	3	1658	99.58
25	358	dead on J+28	—	358	100.00
28	10940	3	8	10951	99.92
33	772	4	4	780	98.97
34	1199	10	10	1219	98.36
37	291	14	3	308	94.48
					99.53=M

mortality of 19 mites, hence a total of 0.47%.

The efficiency of the treatment in this group was of 99.53%.

3.1.3. Mortality in group 3

The third group was the control group, called "treated control group". Notice was taken of the fact that after each of the four applications with Sherring anti-varroa, the percentage of mites' fall was of 27.34%, 19,56%, 5% and 4.9%.



Group 3 *Table 3*

Control Group, Treatment with Amitraz on JO, J+7, J+14, J+21

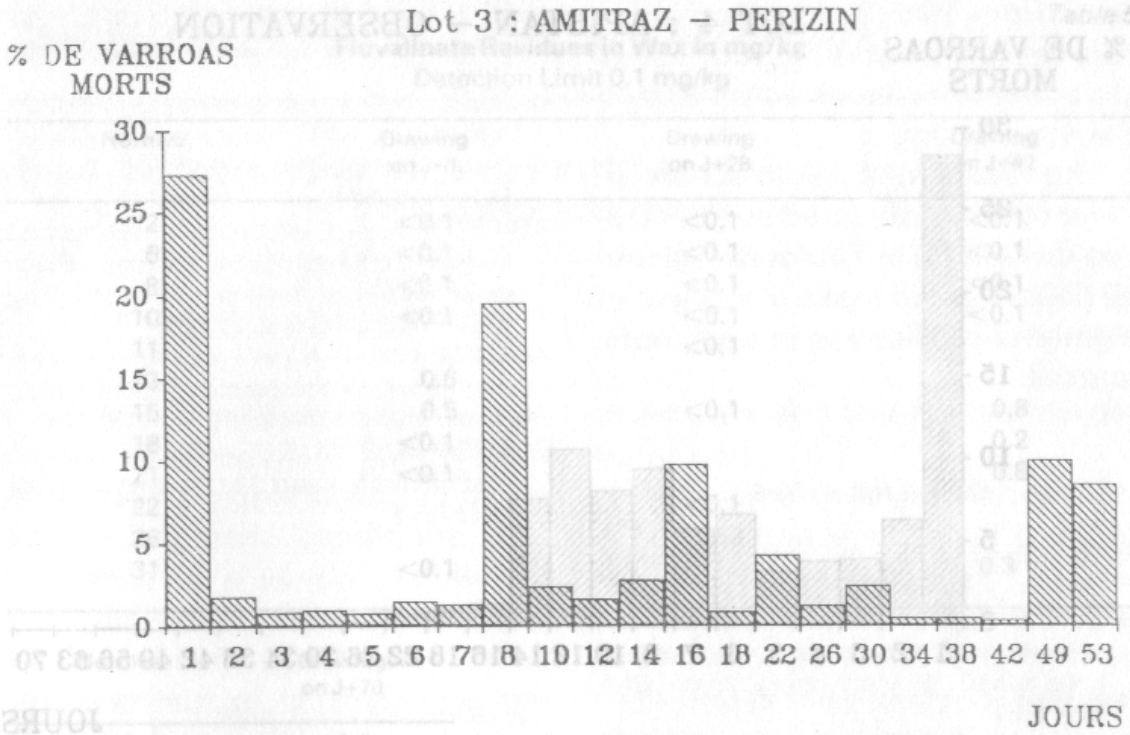
No. colonies	No. dead killed with Amitraz 4 applications from J+8 to J+42	No. dead *Varroa* Control treatment with Perizin		Total no. of dead *Varroa*	Efficiency %
		Application 1	Application 2		
7	4749	11	10	4770	99.56
8	1316	175	135	1626	80.93
10	5510	1004	1032	7546	73.02
18	3223	690	372	4285	75.22
22	1548	10	32	1590	97.86
27	1920	590	477	2987	64.28
30	1062	2	1	1065	99.72
36	264	56	65	385	68.57
38	728	6	20	754	96.55
40	688	31	55	774	88.89
					81.48=M

The other two additional treatments with Perizin caused two additional falls of 9.99% after the first treatment and 8.53% after the second one.

The efficiency of Sherring (Amitraz) treatments did not go beyond 81.48%.

3.1.4. Mortality in group 4

The fourth group was treated like groups 1 and 2 with Apistan, but the observations were made until day J+70. When the strips were removed, 172 mites fell. The efficien-



Group 4 *Table 4*

Treatment with 2 Strips Apistan/Hive, for 6 Weeks

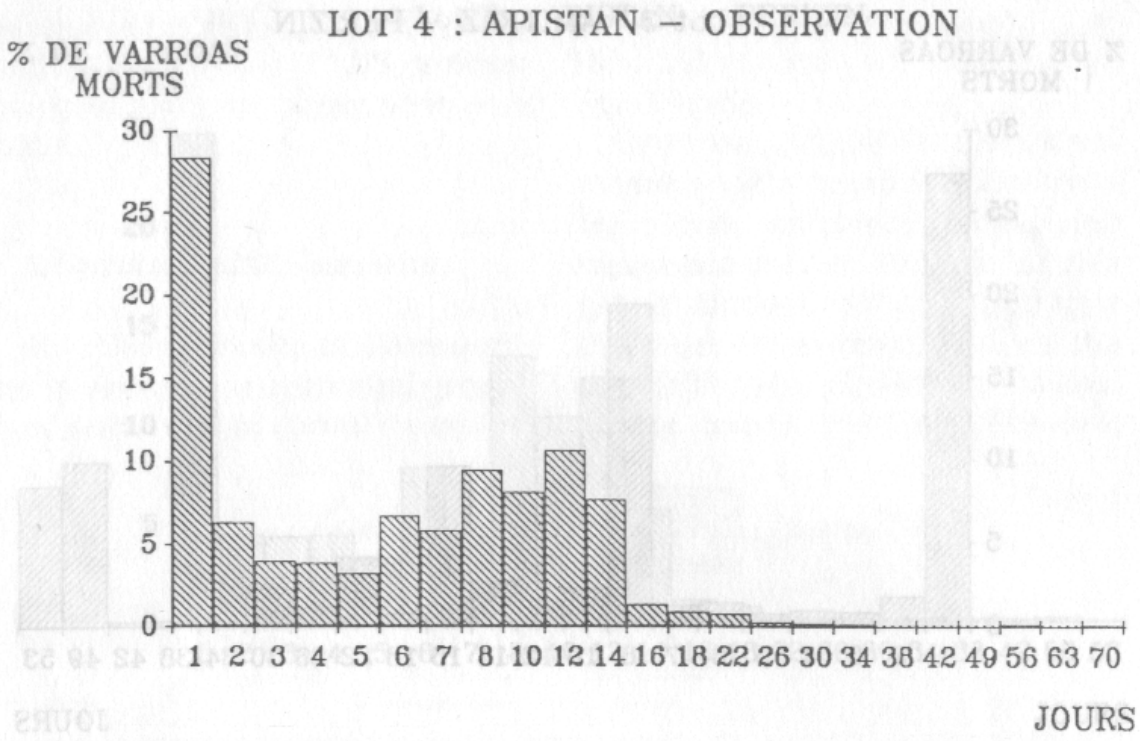
No. colonies	No. dead *Varroa* between J+1 and J+42	No. dead *Varroa* between J+43 and J+70	Total no. of dead *Varroa*	Efficiency %
2	256	20	376	92.75
9	10481	23	10501	99.78
11	3569	8	3577	99.78
13	2960	12	2972	99.60
14	1148	23	1171	98.04
15	2008	13	2021	99.86
19	2907	38	2945	98.71
24	997	14	1011	98.62
32	1886	11	1897	99.42
39	1426	10	1436	99.33
				99.38=M

cy of this treatment with Apistan was of 99.38%

The possibility of a reinfestation is not out of the question.

3.2. Honeybees' mortality

For the four groups, the dead honeybees in front of the hives were: 2512, 2650, 2874 and 2640 between day J–3 and J+42 for populations of about 150,000 and 200,000 honeybees/group. This mortality corresponds to a normal percentage. At the same time, an increase of mortality was noticed in group 3 follow-



ing each stage of the Sherring anti-varroa treatment. It is possible that this mortality is due to the aerosol treatment.

The measure of closing queens in cages seems to cause a disturbance that ends in the following days with a mortality increase in all the groups. The system of counting dead honeybees was not the best but enough to evaluate possible anomalies.

3.3. Changes in behaviour

Throughout the experiments, no behaviour changes were noticed as regards brood rearing or the forage, except for one colony (no. 25) which was weak when the experiments started, and died during the treatment.

3.4. Analysis of Fluvalinat residues in wax and honey

28 out of those 50 samples of wax were analysed in C.P.G. with detection limit of <0.1 mg/kg. The results are given in table 5.

There are two aberrant results for hives 13 and 15 where Fluvalinate was found before the treatment which couldn't be explained satisfactorily.

In the case of honey and in spite of the extreme sensitivity of C.P.G. method with a detection limit of <10μg/kg, those 30 samples analysed from 50 yields never showed Fluvalinat residues.

In the case of wax, if consumed, the quantity of residues is beyond the daily dosage approved in human consumption.

3.5. Mortality Braula coeca

The *Braula* parasites present on honeybee colonies were killed during the first days of using the strips in the colonies. No countings

Table 5

Fluvalinate Residues in Wax in mg/kg
Detection Limit 0.1 mg/kg

No. hive	Drawing on J−1	Drawing on J+28	Drawing on J+42
2	<0.1	<0.1	<0.1
6	<0.1	<0.1	<0.1
8	<0.1	<0.1	<0.1
10	<0.1	<0.1	<0.1
11		<0.1	
13	0.6		
15	0.5	<0.1	0.8
18	<0.1		0.2
21	<0.1		0.8
22		<0.1	
26		0.3	
31	<0.1		0.3

No. hive	Drawing on J+70
2	<0.1
11	<0.1
15	<0.1

were made, but we confirm the good efficiency of Apistan on *Braula,* fact mentioned in previous communications (Apimondia Symposium in Zagreb, 1986).

4. Remarks

This clinic experiment allowed us to notice (groups 1, 2 and 4) a remarkable cohesion of the efficiency of anti-varroa treatment with Apistan during 42 days period and in *the presence of brood.*

— an efficiency situating between 99, 38 and 99.72%.

— a remarkable quality of the molecule of being undamageful to queen, male and worker honeybees.

— total absence of Fluvalinat residues in honey and inferior quantities to those that could be tolerated by D.A.D. (daily approved dosage), in wax.

— a superior application method of the treatment, as compared to other put at the disposal of beekeepers (except for BAYVAROL).

Through its conception Apistan covers all the cases determined by the developmental span in honeybees and *Varroa* mites.

The impossibility of rendering obvious a 100% efficiency of the treatment within such clinical experiment, may be caused by the permanent reinfestation due to drones' and forages' wandering from the neighbouring hives.

The very bad ecoclimatic condi-

tions from 1988 didn't allow us to take in consideration the honey yield from the hives submitted to the experiment.

The results of these tests are similar to those obtained by Prof. Ducos de Lahette in Toulouse. All these papers could make the object of a general publication in a scientific journal.

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