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Original article

## Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy)

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**Summary** — The effectiveness of Apistan® (Sandoz) was tested in 8 apiaries (78 colonies) in some districts of Lombardy (north Italy) during 1993. The strips were left for 22 d in colonies in which the queens were caged inside the hives. On the 24th day, a treatment with 5 ml/comb of Perizin® was given. The fallen mites were recorded every 8 d during Apistan® treatment and 2 d after Perizin®. The treatment's average effectiveness varied greatly from apiary to apiary (min 4%, max 89.5%). In Lombardy, where quite a high mortality of colonies (caused by varroosis) was reported by some beekeepers during autumn 1992, the average effectiveness of the treatment with Apistan® was 44.5%. The results indicate different levels of resistance of *Varroa jacobsoni* to fluvalinate.

***Apis mellifera* / *Varroa jacobsoni* / fluvalinate / effectiveness / resistance**

### INTRODUCTION

Since *Varroa jacobsoni* Oud was first detected in Italy (Barbattini, 1981) various products for controlling *Varroa*, such as Folbex VA®, Apitol®, Apivar® and Perizin® have been registered (in that order) by the Italian Ministry of Health. Nevertheless, the strategies and legally adopted drugs have often been complemented or replaced with other products or treatments by operators on their own initiative. Products based on

fluvalinate (Klartan®) and amitraz (Taktic®, Bumetran® and Edrizar®) are not authorized for use in beekeeping but were widely administered using a variety of methods (Frilli, 1989).

In 1989, these practices were largely curtailed by the registration of Apistan®, especially in regions such as Lombardy where the product was freely distributed to all beekeepers. This began in 1990 as part of territorial programmes to combat varroosis.

Since 1992, some beekeepers and representatives of national organisations have voiced doubts about the effectiveness of Apistan® in controlling varroosis, on the basis of evidence from direct observations. In some regions of Italy, cases of colony mortality due to varroosis in apiaries regularly treated with Apistan® have been found. The problem was first officially noted in northern Italy in the district of Bergamo, and confirmed by the Health Department in 1992 (Loglio and Plebani, 1992; Loglio, 1993). During the summer of the same year, a high mortality rate of hives, sometimes of entire apiaries, was recorded in other provinces of Lombardy.

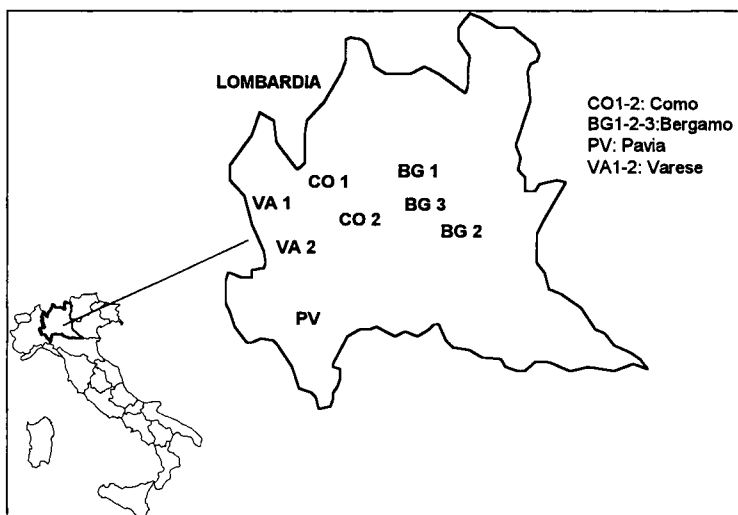
Some of the cases of colony mortality may be ascribed to the incorrect use of the product by beekeepers (Barbattini and Greatti, 1992; Frediani, 1993; Watkins, 1993). However, recurrent reports by reliable operators, together with the spread of the problem and the high mortality rate called for a study to investigate if, in fact,

there was some resistance of *Varroa* to fluralinate.

## MATERIALS AND METHODS

Tests were conducted in 1993, between the end of April and the beginning of June, on 78 hives in 8 different apiaries situated in Lombardy (fig 1). Certain districts of Lombardy were chosen to carry out the tests. There were 2 reasons for this choice: 1) a programme of varroosis control, based on the use of Apistan® alone and supported by special public funds had been conducted in these areas for 3 years; and 2) high colony mortality rates had been observed in these regions.

In the previous year (1992), 4 of the 8 apiaries had been treated exclusively with Apistan® (group A, fig 1); the other 4 were treated with Apistan® and either amitraz or Perizin® (group B). Hives in Emilia-Romagna were initially included as a control group, but the average number of fallen *Varroa* mites detected with the treatments was lower than 10 and these hives were therefore omitted.



**Fig 1.** Location and situation of the apiaries in which the tests were carried out. Group A (VA1, CO1, BG1, BG2): colonies treated exclusively with Apistan® in 1992 in areas where hive mortality for varroosis was recorded; group B (CO2, PV, VA2, BG3): colonies treated in 1992 with Apistan® and Perizin® (CO2, PV) or with Apistan® and amitraz (VA2, BG3), in areas where hive mortality for varroosis was recorded.

For this test, only colonies with similar amounts of brood, bees and stored honey were chosen. Two strips of Apistan® (Lot No 91120191, produced in USA in December 1992) were placed in each hive the first day of the test (day 0) and left for 22 d.

Egg laying was interrupted from day  $t$  until day  $t + 16$  to determine the number of mites surviving after treatment with a systemic product that, to be fully effective, requires the absence of capped brood. For this purpose, the queen of each hive was placed in a queen-cage, placed near the brood area of a central comb. Two days after the removal of the Apistan® strips ( $t + 24$ ), when no sealed brood was present in the colonies, a Perizin® treatment was given to each colony by delivering 5 ml of emulsion to each honeycomb crowded with bees.

Every 8 d during the treatment with Apistan® and 2 d after the treatment with Perizin®, we counted all the mites captured on the paper sheets smeared with vaseline at the bottom of the hives. The effectiveness of the Apistan® treatment was calculated according to the formula:

$$\% \text{eff} = \frac{\text{fallen mites with Apistan}^{\circledR}}{\text{fallen mites with both treatments}} \times 100$$

The average effectiveness of each apiary was calculated to show the situation of the whole population.

The intense activity of the bees due to favourable environmental conditions (daytime temperatures always greater than 15°C in all the tested areas, length of the photoperiod and abundant blooming) during the period in which the tests were conducted, allowed optimal distribution of the active compound within the colonies (Milani and Barbattini, 1988; Borneck and Merle, 1989; De Ruijter and Van Den Eijnde, 1989; Llorente Martinez, 1989). The interruption of brood production further facilitated contact of fluvalinate with the mites. For this reason, the time the strips remained in the colonies was reduced from the 6–10 weeks recommended by the company to 22 d. The intense nectar-collecting activity during the test reduced the risk of reinfestation through robbing. Furthermore, it is possible that some mites that survived the treatment with Apistan® could also have survived a single treatment with Perizin® (Milani and Barbattini, 1988). Thus, the data of Apistan® effectiveness could be overvalued in some cases.

## RESULTS

The hives showed notable differences in the rate of infestation. This varied from a minimum of 1 (CO2, VA2) to a maximum of 5 700 (BG2) mites per colony (table I). Even within the individual apiaries, the degree of infestation was very variable, with an SD often higher than the mean (CO2, VA2). A notable variability in the efficacy values was observed in the colonies of the apiaries of both groups (fig 2). This was particularly evident in the VA1 apiary (min 0%, max 99.1%) and in the PV apiary (min 0.6%, max 92.6%). Only in 16 of the 78 colonies tested yielded an effectiveness greater than 95%.

In the apiaries of group A the effectiveness ranged from a minimum of 4% in BG1 (with values between 1.1 and 8%) to a maximum of 64.1% in BG2 (24.2–98.8%). In group B, a lower average effectiveness was recorded in the PV (mean 10.4%, min 0.6%, max 92.6%) and BG3 apiaries (mean 43.1%, min 25.2%, max 53.9%) compared to that obtained in the CO2 (mean 89.5%, min 52.6%, max 100%) and VA2 apiaries (mean 67.4%, min 59.3%, max 100%)

## DISCUSSION

The average efficacy by apiary was lower than that reported by both Italian (Arculeo *et al*, 1989: 100%; Barbattini *et al*, 1989: 92.3%; Frilli *et al*, 1992: 92.3%; Greatti *et al*, 1992/93: 99.7%; Arculeo *et al*, 1993: 92.7–98.7%) and foreign authors (Llorente Martinez *et al*, 1989: 99.33%; Borneck and Merle, 1990: 99.38–99.72%; Ferrer-Dufol *et al*, 1991: 100%). According to Lendaro and Tonelli (1985), an adequate therapeutic efficacy must be more than 95%, a value which was found in only 20.5% of the tested colonies distributed evenly throughout the apiaries.

**Table I.** Average number of fallen mites with Apistan® and Perizin® treatments.

	No of fallen mites							
	Group A apiaries <sup>a</sup>				Groupe B apiaries <sup>a</sup>			
	VA1 (5)	CO1 (10)	BG1 (7)	BG2 (12)	CO2 <sup>b</sup> (14)	PV <sup>b</sup> (10)	VA2 <sup>c</sup> (9)	BG3 <sup>c</sup> (11)
<i>Apistan</i> ®								
Mean	78	138	52	1 117	68	39	14	304
SD	80.8	109.4	72.3	659.9	133.4	29.3	19.4	274.6
Min/max	0/196	63/430	6/207	186/2 200	1/474	3/84	1/64	42/1 003
<i>Perizin</i> ®								
Mean	124	222	1 257	625	8	332	6	401
SD	123.3	95.5	810.1	978.7	16.3	256.3	14.8	271.7
Min/max	1/289	78/361	536/2 959	10/3 500	0/62	3/706	0/44	39/892
<i>Total</i>								
Mean	202	360	1 309	1 742	76	371	20	705
SD	69.3	186.1	875.2	1 406.2	138.7	265.2	33.7	529.1
Min/max	105/294	183/791	542/3 166	246/5 700	1/476	7/759	1/108	81/1 895
<i>Percentage effectiveness of Apistan</i> ®								
Min/max	0/99.1	22.8/61.2	1.1/8	24.2/98.8	52.6/100	0.6/92.6	59.3/100	25.2/53.9

Average effectiveness of the treatment with Apistan® referred to the apiary. The apiaries are subdivided in groups and localities. <sup>a</sup> Abbreviation for apiary with number of colonies in parentheses; <sup>b</sup> Apiaries were treated with Perizin® in addition to Apistan® in autumn 1992. <sup>c</sup> Apiaries were treated with amitraz in addition to Apistan® in autumn 1992.

These observations of individual colony variation, together with the differences in the average effectiveness obtained in the apiaries, provide evidence for the presence of strains of *V jacobsoni* resistant to fluvalinate in the apiaries located in Lombardy. Additional support for this conclusion comes from observations carried out by Milani (1994) on mites from Bergamo apiaries (close to BG1, BG2 and BG3 apiaries). The resistance of the mites to fluvalinate was tested *in vitro* on paraffin disks. The results showed a LD<sub>50</sub> at least 10 times higher than that recorded by mites collected in apiaries

in Friuli (north-east Italy), where colony mortality in apiaries treated with Apistan® has not been reported.

Many elements lead us to predict that the phenomenon may develop and spread rapidly. The data of this study must be a matter of serious concern to all beekeepers. There is a pressing and urgent need to verify the effectiveness of Apistan®, throughout the shared range of *V jacobsoni* and *A mellifera*, as part of an acaricide-resistance management plan to safeguard beekeeping from serious damage in the future.

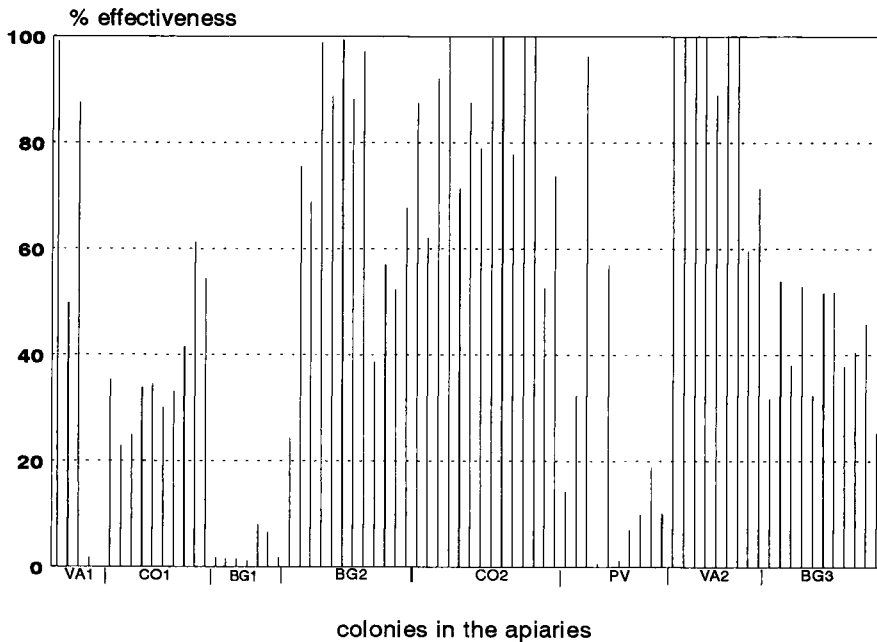


Fig 2. Effectiveness of treatment with Apistan® in single colonies by apiary.

## ACKNOWLEDGMENT

The strips of Apistan® were provided by Federazione Apicoltori Italiani.

**Résumé — Inefficacité du traitement à l'Apistan® contre *Varroa jacobsoni* Oud en plusieurs régions de Lombardie (Italie).** Dans plusieurs régions d'Italie on signale depuis quelques années de fortes mortalités d'abeilles dues à la varroose dans des colonies traitées à l'Apistan®. Afin d'étudier ce phénomène, 78 colonies réparties en 8 localités de Lombardie (fig 1) ont été traitées à l'Apistan® durant 22 j. Lors du traitement on a procédé à un blocage de la ponte en encageant les reines. Les acariens tombés sur les plaques de dépistage ont été dénombrés tous les 8 j. Deux j après le retrait des lanières d'Apistan® un traitement de contrôle au Perizin® a été effec-

tué. La faible efficacité générale du traitement (tableau I) et les grandes différences entre ruchers mais aussi entre ruches d'un même rucher (fig 2) prouvent la présence de souches de *V jacobsoni* résistantes à l'Apistan® et leur diffusion au sein des populations de Lombardie.

***Apis mellifera* / *Varroa jacobsoni* / efficacité traitement / fluvalinate / sensibilité-résistance**

**Zusammenfassung — Unwirksame Behandlung der Varroose durch Apistan® in einigen Provinzen der Lombardei (Italien).** In einigen italienischen Gegenden wurden seit mehreren Jahren hohe Bienenverluste durch die Varroose gemeldet. Die betroffenen Völker waren mit Apistan® behandelt worden. Zur Untersuchung dieser Berichte wurden an 8 verschiedenen

Orten in der betroffenen Region Lombardie insgesamt 78 Völker mit Apistan® behandelt (Abb 1). Die Behandlungsdauer betrug 22 Tage. Zu Beginn der Behandlung wurden die Königinnen gesperrt, sodaß am Ende der Behandlung keine Brut mehr vorhanden war. Während der Behandlung wurden alle 8 Tage die abgetöteten Milben auf den Bodeneinlagen ausgezählt. Zur Bestimmung des verbleibenden Befalls wurden die Völker zwei Tage nach Entfernung der Apistanstreifen mit Perizin® behandelt. Der insgesamt niedrige Behandlungserfolg (Tabelle I) und die grossen Unterschiede zwischen den Bienenständen, aber auch zwischen den einzelnen Völkern eines Standes (Abb 2) weisen auf das Vorhandensein und die Ausbreitung von gegen Fluvalinat resistenten *Varroa jacobsoni*-Linien in der Lombardei hin.

### ***Apis mellifera* / *Varroa jacobsoni* / Fluvalinate / Wirksamkeit / Resistenz**

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